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KINETICS OF SINGLE ORGANIC MOLECULES IN SOIL SOLUTIONS

by

Carlos Maxwell Monreal-Sepulveda

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF Doctor of Philosophy
IN
Soil Microbiology and Biochemistry

Department of Soil Science

Edmonton, Alberta
Fall 1987

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Date SEPTEMBER 18, 1987

Dedicated to the lives of those like *Jose Amigo* who died searching for truth, love, justice and peace.

ABSTRACT

Soluble-C dynamics were studied under non-perturbed (ng ^{14}C-cystine added) and perturbed (mg ^{14}C-glucose added) soil conditions. Chernozemic, Luvisolic and one allophanic soil (Andept) were incubated with labelled substrates for intervals up to six hours. Initial characterization of solution free amino acids in soils at steady-state showed diverse and low amino acid content.

Kinetic analyses and modelling tested conceptual models of soluble-C dynamics. A four compartment model described cystine dynamics in non-allophanic soils at steady-state. The model involves the solution, adsorbed, microbial cytoplasmic amino acids and intracellular proteins. Soil solution cystine turns over in seconds, the adsorbed and cytoplasmic cystine in minutes and the protein-C in hours. Reactions transfering cystine-C are adsorption, desorption, microbial uptake, protein synthesis and oxidation of cytoplasmic and protein carbon. Adsorption retards cystine mobility but does not prevent its utilization by microbes. In a perturbed soil, excess glucose-C is incorporated into intermediates and macromolecules of microbial metabolism.

Cystine dynamics vary in soils with different pedogenic processes and perturbations. Greater $^{14}CO_2$ evolution from virgin Brown than from Dark Brown and Black Chernozemic soils was associated with faster cycling of solution cystine in the former soil group. Cytoplasmic carbon is the main source for CO_2 evolution in cultivated samples whereas protein-C is the main CO_2 source in virgin samples. Addition of amendments decreased the amounts of substrate-C stabilized as proteins in allophanic and non-allophanic soils. The latter molecules behaved like short-lived proteins.

For the Andept soil, two cycling scenarios pertained. The first includes solution, adsorbed, stabilized and one biontic pool similar to microbial proteins of non-allophanic soils. Stabilization to active organoallophanic surfaces rendered some cystine unavailable for microbial use. The second scenario shows no abiotic stabilization. Plant residues changed the soil internal architecture and shifted the center of microbial activities from the organoallophane-solution interface to the surface of decomposing residues. Under such conditions, cystine dynamics is controlled only by microbial metabolism.

The present kinetic models defined physiological controls (biontic carbon flow) of soluble-C dynamics by integrating experimental observations with knowledge of different sciences.

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1. INTRODUCTION

Soil is a complex system, formed by interactions of several natural factors. It is composed of different organic and inorganic components organized into heterogeneous matrices. In addition, soils are unique dynamic systems undergoing continuous transformations and interactions between biontic[1] and abiotic components. These reactions are catalyzed mainly by biological and chemical agents but also to a limited extent by physical agents.

In most soils the organic component constitutes the main reservoir of C,N,P,S and only a small proportion of the organic matter is present in a free state (Harter, 1977; Campbell, 1978; McGill *et al.* 1981). This soluble and labile fraction is susceptible to reactions such as formation of organo-metallic complexes and adsorption. These reactions in turn are dependent on properties of the participating minerals and organics (Harter, 1977; Theng, 1973). Reactions between mineral and organic moieties may affect soil genesis, plant nutrient availability, and the type of plant or microbial taxa that can exist in any given situation. Soil organic matter transformations supply nutrients for plant growth. Plant biomass production is intimately associated with and dependent on energy and nutrients flowing through active soil microbial taxa. The directions of element flows are regulated in single microbial species by various enzymes, allosteric effectors and the energy charge of the cell (Atlas, 1984). In soils these factors can be modified by the solution composition, mineral phase and reactions between biontic and abiotic pools.

Past research has produced valuable basic information about organics, minerals and microorganisms in many areas of soil science and microbiology. Most of these studies, however, have embraced experiments where soil and microbial components were studied in isolation and independently from others (Harter, 1977; Theng, 1973; Anraku, 1980). Improper integration of such knowledge can result in dissemination of contradictory information creating confusion rather than clarification of processes and mechanisms involved in soil organic matter changes (Stotzky, 1986). Hence, new approaches are needed to advance knowledge of soil ecosystems. To increase the understanding of controls[1] of organic matter dynamics, it is essential to integrate knowledge of past research with information obtained *in vivo* from perturbed and non-perturbed soil systems. Analytical chemistry, biochemistry and mathematical modelling complement one another in achieving this goal (Shipley and Clark, 1972; Bassingthwaighte and Winkle, 1982). Achievement of this goal will assist man to manage soils and improve their general quality.

---

[1] biontic term used to identify all active non-photosynthetic prokaryotes and eukaryotes capable of metabolizing glucose and cystine in soils

Within this context, soluble organic fractions such as free amino acids and glucose can be useful in the study of controls of soil organic matter dynamics because they are molecules that satisfy the energy and other elements required by microorganisms (Anraku, 1980). Use of such molecules within "*in vivo*" soil systems can provide further insights and augment knowledge of biontic and abiotic soil components controlling the supplies of nutrients to plants.

Thus, the objectives of these studies were to:

a. Study the controls on free cystine and glucose dynamics *in vivo* perturbed and non-perturbed soil systems.

b. Study the expression of such controls in soils experiencing a range of pedogenic processes, and perturbations such as management histories and agronomic practices.

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---

[1] Control of C flowing through biontic or abiotic soil components. Control does not refer to control of microbial enzymatic reactions at the gene level

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2. CENTRIFUGAL EXTRACTION AND DETERMINATION OF FREE AMINO ACIDS IN SOIL SOLUTIONS BY TLC USING TRITIATED 1-FLUORO-2,4-DINITROBENZENE[3]

2.1 INTRODUCTION

Several methods to remove the soil solution have been tested using the principles of suction, pressure, miscible and immiscible displacement, and centrifugation (Adams, 1974). The ionic composition of soil solutions separated by column displacement, sample centrifugation, and by immiscible displacement with CCl_4 were unaffected by the method used (Adams *et al.*, 1980).

Soil solution is a reactive medium where chemical, biochemical and physicochemical reactions occur. Diverse techniques have been used in the past to identify organic molecules in soil extracts, especially free amino acids. Sowden and Parker (1953), were unable to detect free-NH_2 groups as their DNP derivatives of organic fractions isolated from soil. Other studies on the free amino acid pool of soils have used paper and column chromatography (Putnam and Schmidt, 1959; Paul and Schmidt, 1960). Automatic amino acid analyzers, gas chromatography and high performance liquid chromatography, among others, permit, in principle, sensitive and reliable methods for separation and quantification of amino acids and other organic molecules of biological origin (Chan and Ridley 1966; Ivarson and Sowden, 1970; Pellizari *et al.*, 1971; J.D. Helgeland unpublished Ph.D. thesis, University of Alberta, 1975; Hancock *et al.*, 1979).

Some of these techniques require prior removal of contaminants (carbohydrates, organic acids, salts) using ion exchange columns. This purification step may result in incomplete recovery of mixtures of basic, neutral and acidic amino acids (Pellizzari *et al.*, 1971). Cleaning of the sample extends the time of analysis and studies with labelled amino acids require special devices to collect radioactive fractions. Also, several sources of error arise in the determination of specific radioactivity of amino acids isolated by ion-exchange chromatography (Gaitonde and Nixey, 1972).

---

[3] A version of this chapter has been published. C.M. Monreal and W.B. McGill (1985). Soil Biology and Biochemistry, 17: 533-539.

Dinitrophenyl amino acids, a technique used by Sanger (1945), to analyze the free amino groups of insulin was adapted by Beale and Whitehead (1961) for quantitative purposes. This technique can detect 0.2 μg amino acid (Brenner *et al.*, 1969), thus providing an alternative method of separation and quantification of free amino acids. The advantages of this method are lower costs, elimination of a cleaning step and ease of obtaining radioactive fractions from TLC plates.

The objectives of this study were: (i) to test a centrifugation method of removing the soil solution; (ii) to examine the feasibility of using TLC fractions of ^{3}H-DNP amino acids to separate and quantify free amino acids from soil solutions; and (iii) to examine amino acid concentrations in solutions from diverse soils.

2.2 Materials and Methods

2.2.1 Soils

Soils with different management histories were used (Table 2-1). Samples were taken from cultivated fields and from adjacent virgin sites in the fall of 1981 and subsequently stored at < 3°C prior to use.

2.2.2 Incubation of soil samples

A bulk soil sample was sieved to < 2mm. Two 10 g samples (oven dry basis) were equilibrated by incubating them at -33 kPa soil moisture potential and 20±1°C for 1 month.

2.2.3 Soil Solution

Each 10 g soil sample was distributed among 6 centrifuge cells (Mandel, model CCI) with a microcentrifuge tube (1 ml) as the receptacle. A micropore filter, 0.2 μm, cut to size (1.4 cm diameter) was used as the filter. The soil solutions used for chromatography were combined for each soil after centrifugation at 10,000 g for 5 min at 0.5°C in an angle head

IEC B-20A high speed centrifuge. The volume of solutions obtained was measured with a graduated 1 ml glass pipette (± 0.01 ml) and with an Eppendorf micropipette (graduated 10 - 100 μl). The extracted soil solutions were then transferred to a 10x75 mm test tube placed in an ice bath or frozen until analysis.

2.2.4 Derivatization and extraction of DNP amino acids

Each soil solution sample was spiked with 5 μl of a standard mixture containing 1.25 to 2.50 nmoles μl^{-1} of each amino acid (Sigma) and the pH of the solution was adjusted to 8.5 - 9 by adding 1% w/v of potassium borate. Deionized water (200 μl) was then added to a sample containing only the standard mixture of amino acids. Under exclusion of direct light, 100 μl of 1-Fluoro-2,4-[3,5-^{3}H] dinitrobenzene (New England Nuclear) reagent were added. This reagent was prepared by adding 300 μl of 1-Fluoro-2,4-[3,5-^{3}H] dinitrobenzene with a specific activity of 651 GBq $mmol^{-1}$, and 130 μl of 1-Fluoro-2,4 dinitrobenzene (98% pure by GLC obtained from Kodak) into 10 ml of 98% ethanol.

Each test tube was wrapped with foil, mixed vigorously and incubated at 60°C in a water bath for 30 minutes. Excess dinitrofluorobenzene was removed by extracting twice with 1 ml of peroxide-free ether and removing the organic phase with a Pasteur pipette. The remaining aqueous phase was acidified with 100 μl of diluted HCl(1:1), and separated into acid-insoluble and acid-soluble DNP amino acids. These were then extracted in separate tubes 6 times with 1 ml peroxide-free ether and 6 times with 1 ml ethyl acetate-n-butanol (3:1) solutions, respectively, according to the method of Brenner *et al.* (1969). The organic solvents containing the DNP amino acids were evaporated and the yellow residues in the test tubes dried under vacuo.

2.2.5 Chromatographic separation and quantification

The dried DNP derivatives were dissolved in 50 μl of 50% acetone and homogenized with a vortex mixer. Ten μl of each solution were applied quantitatively with a Desaga micropipette to two separate 20x20 cm silica gel K6F plates (Whatman). The spots were dried under a mild current of air for two minutes before development.

Ascending two-dimentional chromatography was used for acid insoluble ether-extractable DNP amino acids. The solvent system in the first direction (18 cm) was the toluene system of Brenner *et al.* (1969): Toluene:pyridine:ethylene chlorhydrin:0.8M ammonium hydroxide (50:15:30:30). The solvent system in the second dimension (18 cm) was: chloroform:benzyl alcohol:acetic acid (70:30:3). Development and drying conditions followed Brenner *et al.* (1969).

Acid-soluble DNP derivatives extractable with ethyl acetate-n-butanol (3:1) were separated using one dimensional ascending TLC with n-butanol:30% ammonium hydroxide (80:20) as the solvent system.

The layers were inspected under a long wave U.V. lamp within one hour after development. Dark spots corresponding to DNP-derivatives were traced, and then scraped into 15 ml of Scintiverse I scintillation cocktail (Fisher). The ^3H activity of DNP-derivatives was determined by liquid scintillation counting using a Searle Isocap 1300 system after dark adaptation. Efficiency of counting was calculated using the sample channels ratio method.

2.2.6 Calculations

The content of each amino acid was calculated as follows:

$$AA = V \times A \times (B-C)/C \times WA/WR \times 100/S$$

where:

AA = μg amino acid 100g^{-1} soil.

V = Volume of standard mixture (5 μl).

A = concentration of amino acid in the standard solution (μg) μl^{-1}.

B = net disintegration min^{-1} in soil solution.

C = net disintegration min^{-1} in the standard mixture.

WA = water added (ml).

WR = water recovered (ml).

S = weight of soil (g).

2.3 Results

Experiments were conducted to examine the effect of centrifugal force and time of centrifugation on the quantity of water soluble organic carbon (WSOC) removed from soil samples (Table 2-2). The volume of water extracted, and the WSOC content of the solution were not significantly different with increasing centrifugal force beyond 10,000 g, or time of extraction beyond 5 min. Centrifugation (10,000 g, 5min) of the cultivated Malmo SiCl soil removed 0.16 ml of solution representing 53% of the added water. The WSOC content ranged from 58-65 μg ml^{-1} for the different speeds and extraction times (Table 2-2), and the NH_4^+-N content ranged from 3.2-4.4 μg g^{-1}.

Two dimensional chromatography permitted separation and identification of 19 DNP-derivatives of naturally occurring soil amino acids; 13 appeared as single spots and 6 appeared in three spots containing two amino acids each (Figure 2-1). Table 2-3 contains R_f values obtained in the first dimension (directly determined) and second dimension (indirectly obtained). They are consistent with those reported by Brenner *et al.* (1969).

A separate experiment was carried out to optimize recovery of DNP amino acids. The dry residues containing standard mixtures of amino acids were taken up in either 50 or 100 μl of acetone before being applied to the silica gel layer. The net dpm of tritium was proportional to the amount of each amino acid added regardless of whether it was taken up in 50 or 100 μl of acetone (Table 2-4). Therefore, 50 μl of acetone was sufficient to dissolve all DNP derivatives in the dry residue, and spotting 10 μl of this solution accurately transferred measurable quantities of the DNP-amino acids to the layer.

The soil solutions of four separate samples of the Vauxhall and the Malmo soils were extracted and chromatographed to test the hypothesis that free amino acids in the soil solution can be quantitatively recovered through TLC as their DNP derivatives and that co-extracted materials did not interfere in the analysis. Only two soil solution samples from each soil were spiked with 5 μl of standard amino acid solution. The net dpm values of DNP-amino acids of spiked and non-spiked soil solutions were compared with the standard solution on a one to one basis. In both soils, the difference between spiked and non-spiked samples was equal to the activity of the spiking solution measured separately (Table 2-5).

Nine soil samples representing a range of soils and cultural conditions (Table 2-1) were incubated for 30 days to determine if there was any difference in free amino acid content among them (Table 2-6). Total free amino acid content in cultivated and virgin soil samples within the Chernozemic order, decreased in the following order: Brown > Dark Brown > Black. Virgin samples contained more free amino acids than cultivated samples and with the Luvisolic cultivated soil samples, the two year rotation system of grain-fallow contained more free amino acids than those from the 5 year rotation system of grains and legumes.

Air drying and to a greater extent, oven drying of the Malmo soil increased the amount by as much as 10 fold and altered the distribution of single amino acids (Table 2-6). In general, the group aspartic acid-glutamic acid was present in most soils. Arginine, a basic amino acid, was detected only in the Brown and the Dark Brown soils. Phenylalanine, threonine, and alanine occurred most frequently followed by valine, serine, proline, glycine and the leucine complex. Sulfur containing amino acids were found less frequently and at lower concentrations. The content of individual amino acids for the incubated soils was always $< 100\ \mu g\ 100\ g^{-1}$ soil.

No DNP derivatives were detected in the soil solution of the Andept soil. Moreover, a negative recovery was obtained for every amino acid after chromatography.

2.4 Discussion

The need to collect the soil solution rapidly before it undergoes alterations has been recognized (Soon and Miller, 1977). This is even more important when biological reactions in the soil system are to be measured during short time intervals.

Results indicated the soil solution content of WSOC was not affected by different gravitational forces applied to the soil sample. This indicates that microbial cells, which could have been "squeezed" in between soil colloids and minerals, do not seem to be destroyed or to release NH_4^+ or C-containing metabolites from free microbial cytoplasmic pools (Anraku, 1980). Variation among the treatments appears to be due primarily to heterogeneity of the soil system.

Detailed examination of the transformations and cycling of soil organic matter through the solution phase requires determinations of the actual concentration of naturally occurring organic compounds such as free amino acids or free carbohydrates. In this regard, TLC was useful and unambiguously separated the DNP-derivatives belonging to the large group of acid insoluble DNP-amino acids, extractable with ether and the acid soluble DNP-amino acids, extracted with ethyl acetate-n-butanol. The solvent systems used did not separate the DNP-leucine group, the di-DNP lysine-tyrosine and the DNP-glutamic-aspartic acids. Alternate solvent systems described by Brenner *et al.* (1969) enable their separation.

The small fluctuation in R_f values indicates good qualitative accuracy. Operating procedures must be rigidly standardised. In this respect it was observed that derivatization was incomplete at a pH < 8.5, whereas at pH > 9.0, degradation products of DNP-amino acids appeared in chromatograms.

The coefficient of variation of recoveries ranged from 0.2 to 7.0%. Quantitatively, therefore, the method is reproducible. The upper limit of separation is about 1.5 μg for some amino acids such as threonine and serine. The lower limit of reliable detection is 0.2 μg of amino acid.

Quantification of free amino acids in the soil solution was more reproducible and identification was easier when unknown samples were spiked with a standard mixture of amino acids containing at least, 0.2 μg of each DNP derivative. This procedure takes into account the possible losses in activity of the ^{3}H-DNP-derivatives that occur during solvent extraction and development. Previous quantitative assays with TLC silica gel G layers have shown total recovery of radioactivity of ^{3}H and ^{14}C applied to such layers (Snyder and Stephens, 1962). The quantitative technique described in this study is different from that presented by Beale and Whitehead (1961) who determined the concentrations of each amino acid using the specific activity of the original ^{3}H-DNFB reagent. To compensate for losses in activity during solvent extraction, they applied a factor based on the loss in activity of a ^{14}C-labelled DNP-amino acid that was derivatized, extracted and chromatographed simultaneously.

The procedure used in this thesis differs from that of Sowden and Parker (1953) who attempted to derivatize the free amino groups by adding dinitrofluorobenzene to the soils and then extracting it. In their study this reagent was probably adsorbed by the soil colloids thereby preventing the derivatization reaction and subsequent extraction of the product with water from the soil. Also, it would be difficult to maintain the optimum pH under such conditions. Paul and Schmidt (1960, 1961) used NH_4OAc and $Ba(OH)_2$ at extractant:soil ratios of 10:1 using 250 g of soil. Gilbert and Altman (1966) used ethanol at an extractant: soil ratio of 2:1 with 25 g soil. The latter two methods remove adsorbed amino acids as well as those in solution. The method in this thesis uses 10 g of soil, extracted at -33 kPa moisture potential, and is specific for free amino acids in the soil solution.

The presence of cystine and complete absence of histidine in solution in these soils is the main difference between these data and earlier reports (Paul and Schmidt, 1961; Gilbert and Altman, 1966; Ivarson and Sowden 1970). Methionine and cystine contents were low and they were found less frequently than other amino acids. This suggests either high turnover rates due to microbial utilization for energy production and biosynthesis (Anraku, 1980), strong association with soil organic or inorganic colloids, or both. Sulfur containing amino acids may be important in linking components of humic acids (McGill and Cole, 1981) and would thereby be in low concentrations in solutions.

Basic amino acids were detected infrequently. This is consistent with their high isoelectric points (Lysine, 10.53; Histidine, 7.59; Arginine, 10.76) (Barker, 1971) and associated protonation of their extra N atoms at the clay surface (Theng, 1973). Such conditions would remove most basic amino acids from solution.

The highest content of amino acids within the Chernozemic order was in the Brown soils. Varying characteristics of the organic matter or different levels of biological activity may be responsible. In this context, Campbell and Souster (1982) reported a higher potentially mineralizable N (N_0 was obtained by kinetic analysis of net nitrate-N accumulation curves) in Brown soils than in Dark Brown and Black Chernozems from Saskatchewan.

Air drying and especially oven drying increased the content of free amino acids in the soil samples. Microbial cells are susceptible to death under extremely adverse environmental conditions (Jenkinson, 1966). In this respect, heat causes more damage than air drying. This flush of amino acids may have a microbial origin, but the contribution of stabilized organic components cannot be eliminated. The dynamic nature of free amino acids in the soil solution is illustrated in these data. The free amino acid concentration was only about 9% as great when the Malmo soil was moist as when it was oven dry. While adsorption is important in retaining amino acids in both cases, microbial activity becomes important only in a moist soil. Hence, the impact of microbial metabolism of amino acids is reflected by these results. The latter further show that free amino acids are present in the solution phase and not only in adsorbed form in several soils. As such they can participate directly in microbial processes; either as substrates, inducers, or chemo-attractants. The concentration of free amino acids in these soil solutions exceed the Ks values for amino acid auxotrophs reported by Pirt (1975). Consequently, they can also provide a suitable source of amino acids to soil microorganisms.

Amino acid utilization does not depend on solution concentration but rather on rate of supply which in part is a function of desorption. The latter process would in turn be a function of microbial activity. Free amino acids may be supplied to the soil solution in three other ways. Burns (1982) proposed that immobilized extracellular enzymes may produce soluble trigger molecules to induce enzyme production by soil microorganisms. Free amino acids would fall within that category. These nitrogen containing molecules may also be released through plant root exudation or through cell lysis.

The negative recovery of a mixture of amino acids from the soil solution of an Andept soil, shows once again the peculiar behaviour of this soil (Monreal *et al.*, 1981). Free amino acids were possibly complexed with metal ions in such a way that they could not be extracted with ether or the n-butanol-ethyl acetate solvents. High extractable Al has been reported for this soil (Espinoza, 1975).

In conclusion, the proposed method effectively and economically separates and quantifies free amino acids in the soil solution of agricultural soils except in those high in extractable metallic ions. Variations in content and distribution of free amino acids were observed in response to soil forming factors and to management. Further research is warranted into the kinetics of amino acid supply to and removal from the solution phase of these soils.

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Table 2-1. Properties and characteristics of the A_1 used in this study.

Classification									
USA taxonomy:	Argialbol						Boralf		Dystrandept
Canadian system:									
Order:	Chernozemic						Gray		
Great Group:	Brown		Dark Brown		Black		Luvisolic		
Location:	Vauxhall[1]		Lethbridge		Edmonton		Breton		Chile
	Management						Rotation		
	C•	V	C	V	C	V	2 y	5 y[3]	Cultivated
Mean annual Precipitation (mm)	325		381		460		457		1300
Temperature (C)	6.1		5.0		1.5		1.7		13.5
Total C %	1.50	2.40	2.30	3.00	7.00	10.80	1.10	2.00	7.30
Total N %	0.13	0.19	0.17	0.25	0.53	0.79	0.10	0.15	0.66
H_2O -33 kPa[2] potential	20	14	23	23	36	50	16	17	80
Texture Sand %	48	58	30	40	20	20	45	39	12
Texture Clay %	14	12	34	35	38	39	18	16	62

[1] At this location, samples were not taken from adjacent sites.

[2] % moisture oven dry basis.

[3] Control plots (5 y = wheat-oat-barley-forage-forage; 2y = wheat-fallow)

• C = cultivated; V = virgin (not cultivated).

Table 2-2. Characteristics of solutions from the Malmo soil extracted by centrifugation. Each value represents an average of duplicated samples. Soils were moistened to -33 kPa potential with 0.36 ml water and the soil solution extracted after 2 hours incubation.

Gravitational force	Time	Volume recovered	Organic Carbon*		NH_4^+-N**	
(xg)	(min)	(ml)	$(\mu g\ ml^{-1})$[1]	$(\mu g\ g^{-1})$	$(\mu g\ ml^{-1})$	$(\mu g\ g^{-1})$
5,000	5	0.13	62 ± 1	22	3.4 ± 0.4	1.2
	10	0.15	63 ± 4	23	4.2 ± 0.4	1.5
10,000	5	0.16	61 ± 4	22	3.4 ± 0.3	1.2
	10	0.17	63 ± 5	23	4.4 ± 0.4	1.6
	15	0.16	62 ± 2	22	4.2 ± 0.1	1.5
15,000	5	0.16	65 ± 7	23	4.0 ± 0.3	1.4
	15	0.17	63 ± 4	23	3.2 ± 0.1	1.2
20,000	5	0.17	58 ± 1	21	4.0 ± 0.2	1.4
	10	0.18	64 ± 4	23	3.6 ± 0.2	1.3

[1] Mean ± standard deviation

* Measured using a Beckman model 915-B soluble C analyser.

** Measured utilizing the Berthelot reaction in a Technicon autoanalyser II.

Table 2-3. R_f-values of the ether and butanol-ethyl acetate soluble DNP-amino acids observed after two and one dimensional ascending chromatography. 12.5 nmoles spotted except 6.25 nmoles for cystine.

	First Dimension ascending		Second Dimension ascending	
DNP-Amino Acids	Rf[2]	sd[3]	Rf	sd
DNP-ALA	16.2	0.8	26.2	0.8
DNP-ARG[4]	13.4	0.7	—	—
DNP-(ASP+GLU)	1.2	0.0	6.4	0.8
Di-DNP-CYS	3.0	0.0	0.0	0.0
DNP-GLY	12.6	0.8	14.0	0.5
Di-DNP-HIS	35.3	1.0	—	—
DNP-(ILEU+LEU)	26.9	0.8	52.6	0.4
Di-DNP-(LYS+TYR)	32.3	0.8	24.7	0.4
DNP-MET	22.3	0.6	33.7	0.7
DNP-PHE	24.6	0.8	36.0	0.0
DNP-PRO	14.1	0.4	29.1	0.8
DNP-SER	7.2	0.3	4.9	0.4
DNP-THR	10.8	0.5	10.5	0.6
DNP-VAL	21.6	0.0	43.6	0.2

[1] $R_f = \frac{\text{distance of spot centre from start point}}{\text{distance of solvent from start point}} \times 100$

[2] Solvent migration was 18 cm in first and second dimension.

[3] Standard deviation of four replicates.

[4] Solvent migration was 10 cm for development of DNP-Arg and Di-DNP-His.

Table 2-4. Standardization of the method for quantitative purposes with a standard solution of amino acids.

DNP-amino acids	5-100-10		5-50-10[1]		10-50-10	
	Amount spotted (μg)	Spot activity (dpm)	Amount spotted (μg)	Spot activity (dpm)[2]	Amount spotted (μg)	Spot activity (dpm)
DNP-ALA	0.112	175 ± 7	0.224	350 ± 15	0.448	694 ± 30
DNP-ARG	0.218	193 ± 12	0.436	396 ± 8	0.872	801 ± 17
DNP-(ASP+GLU)	0.351	191 ± 12	0.702	390 ± 11	1.404	775 ± 21
Di-DNP-CYS	0.150	164 ± 10	0.300	320 ± 21	0.600	656 ± 18
DNP-GLY	0.094	194 ± 12	0.188	380 ± 15	0.376	766 ± 18
Di-DNP-HIS	0.194	111 ± 8	0.388	218 ± 12	0.776	428 ± 12
DNP-(Ileu+Leu)	0.328	377 ± 16	0.656	763 ± 13	1.312	1494 ± 16
dl-DNP-(Lys+Tyr)	0.409	509 ± 18	0.818	1060 ± 12	1.636	1811 ± 18
DNP-MET	0.186	194 ± 8	0.372	389 ± 7	0.744	770 ± 18
DNP-PHE	0.206	281 ± 5	0.412	569 ± 12	0.824	1195 ± 7
DNP-PRO	0.144	360 ± 7	0.288	715 ± 8	0.576	1468 ± 35
DNP-SER	0.131	205 ± 13	0.262	404 ± 20	0.524	816 ± 18
DNP-THR	0.149	151 ± 10	0.298	309 ± 6	0.596	601 ± 16
DNP-VAL	0.146	189 ± 13	0.292	391 ± 14	0.584	798 ± 12

[1] 5-50-10 = 5 - aliquot taken from original standard amino acid solution (μl)
50 - volume of 50% acetone in which dry residue was dissolved (μl)
10 - volume spotted (μl)

[2] dpm = mean value ± standard deviation of four replicates.

Table 2-5. Effect of soil solution on derivatization, separation and counting of ^3H-DNP-amino acids.

	^3H activity (dpm of scraped spots from TLC plates) Soil Solution					
	Vauxhall (virgin)			Malmo (cultivated)		
DNP-amino acids	spiked	not spiked	% recovery	spiked	not spiked	% recovery
Di-DNP-Cys	440	110	103			
DNP-Gly	553	182	98	928	530	105
DNP-(Ileu+leu)				945	170	102
DNP-Phe				601	29	101
DNP-Pro	1112	389	101	1090	361	102
DNP-Ser				778	382	98
DNP-Val	448	49	102	531	126	104

Table 2-6. Free amino acids in soil solutions measured as their DNP-derivatives. Soil samples were incubated at -33 kPa moisture potential and at 20 C for 30 days. Values in μg amino acid per 100 g^{-1} soil ± standard deviation of replicates.

Location	Breton		Vauxhall		Lethbridge		Edmonton				Chile
Amino acid	Gray Luvisol (Rotation)		Brown		D. Brown		Black				Andept
									(Cultivated)		
	2 years	5 years	Culti-vated	Virgin	Culti-vated	Virgin	Culti-vated	Virgin	Air dry	Oven dry	Culti vated
Acidic											
(Asp+Glu)	73±11	14±1	76±15				4±1	24±8	17±3	220±6	- 1± 2
Basic											
Arg			8±4	83±25		91±22			4±1	8±2	-12± 5
His											-21± 3
(Lys+Tyr)[1]	38± 8										- 1± 1
Neutral											
Ala	7± 2	4±1	2± 1	35±10	3± 1	52±15				2±1	- 1± 3
(Cys)2	37± 5		18± 3	34± 9					43±1	- 1± 2	
Gly	11± 2									19±0	- 5±11
(Ileu+leu)		12±1					5±1				- 7± 6
Met		4±2		29± 2						21±2	-10± 1
Phen	22± 4		29±20		95±20	11± 7	10±0	35±5			- 4± 3
Pro			8± 3	28± 7			2±1		10±0	13± 0	- 2± 3
Ser	23± 5		22± 3	44± 5	63±20				9±1	14±0	- 1± 4
Thr	25± 3		15± 6	72±16	69±17	11± 3	3±1				- 2± 5
Val			11± 5	52±15			8±0	4±2	3±1	8±2	-10± 4
Total	262	34	189	472	135	165	32	63	43	348	< 0

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[1] In spite of being a neutral amino acid, tyrosine was classified as basic because it develops together with lysine.

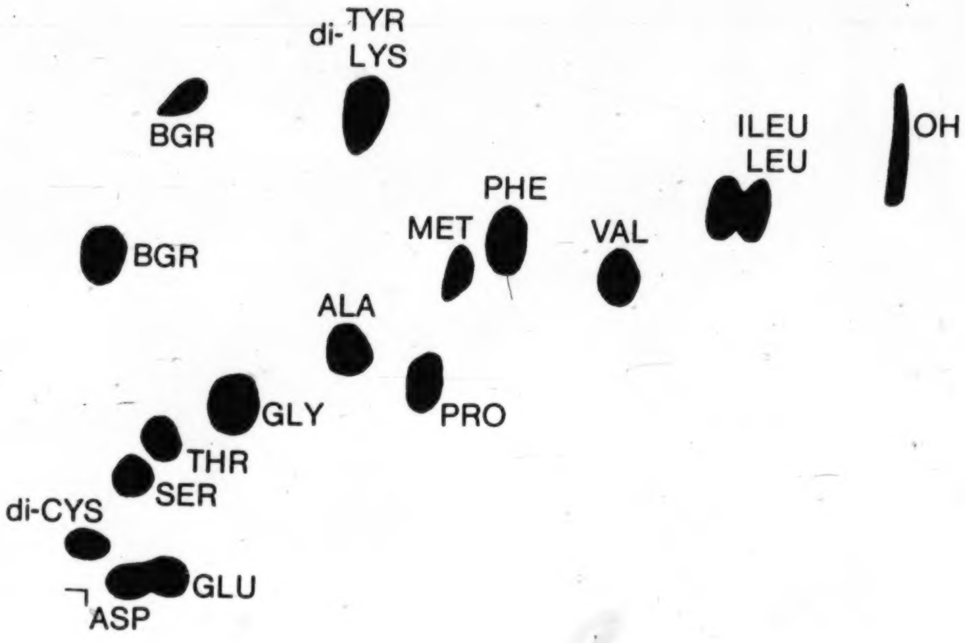
di-TYR
LYS
BGR
ILEU
LEU
OH
PHE
MET
VAL
BGR
ALA
GLY
PRO
THR
SER
di-CYS
GLU
ASP

Figure 2-1. Separation of a mixture of DNP-amino acids from soil solutions after ascending two dimensional chromatography. Solvent systems run 18 cm in both directions. The layer was loaded with 12.5 nmol of each amino acid except 6.25 nmol for cystine.

3. KINETIC ANALYSIS OF CYSTINE CYCLING THROUGH THE SOLUTION OF A GRAY LUVISOLIC AND AN ANDEPT SOIL[1]

3.1 Introduction

Modern soil organic matter studies include simulation modelling as a tool to describe internal soil architecture and biochemical processes. Most models describe the dynamics of complex substrates such as microbial biomass and plant residues over days and weeks (Hunt, 1977; McGill *et al.* 1981, Van Veen *et al.* 1981). On the other hand, release of nutrients to the plant available pool is normally associated with fast (minutes, hours) microbial processes involving organics such as amino acids. Free amino acids have been studied with regard to plant uptake (Skinner and Street, 1954; Wright, 1962), microbial utilization (Soda *et al.* 1964) and adsorption to clay minerals (Putnam and Schmidt, 1959; Theng, 1973), but their cycling through soil solutions, microbial, and adsorbed phases has not been described. Kinetic studies of free amino acids in the soil solution may therefore, provide useful information about control mechanisms affecting availability of nutrients to plants. If kinetic studies are to simulate real soil systems, experimental conditions must use substrates in smaller concentrations than are found in the normal habitat. Distortions of ecosystems are then minimized (Hobbie, 1973).

Complex systems can be described using mathematical formulations based on experimental observations of several components. Such formulations reduce the degrees of freedom in mathematical models and increase their rigor. This assists in devising experiments to elucidate the rate parameters for those parts of the system which are most critical or least known (Bassingthwaighte and Winkle, 1982). Alternatively, completely interconnected systems experience flow in both directions between compartments. They can be described using kinetic compartment analysis of measurements made in one of the completely interconnected compartments (Shipley and Clark, 1972). Kinetic compartment analysis has been successfully applied to mammalian metabolism (Baker *et al.* 1958), plant photosynthesis (Hatch and Osbond, 1976), and human medicine (Bassingthwaighte and Winkle, 1982). Labroue *et al.* (1985), however found such an approach was not well suited to describing mineral-N dynamics in cold soils under field conditions. A similar approach to study controls of dynamics of free amino acids could explain the low amino acid content found in soil solutions (Chapter 2).

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[1] A version of this chapter will be submitted for publication. C.M. Monreal and W.B. McGill (Soil Biology and Biochemistry)

The objective of this study was to describe "in vivo" cycling of ^{14}C-cystine through kinetically homogeneous compartments in two soils by using kinetic analysis and modelling techniques.

3.2 Materials and Methods

3.2.1 Soil

A Gray Luvisolic (Breton L., Alberta) and an Andept soil (Chile) were used in this study. Their properties have been described in Chapter 2.

3.2.2 L-[^{14}C(U)]-cystine

Uniformly labelled cystine was obtained from New England Nuclear, with a specific activity of 8.3 x 10^7 Bq mg^{-1} cystine. Cystine was added at 60 ng g^{-1} soil, which was equivalent to less than 17% of its natural content found in these soils (Chapter 2). The purity of cystine was checked by two dimensional thin layer chromatography using butanol:acetic acid:water (4:1:1) and phenol:water (4:1) as the solvent systems in the first and second directions, respectively. After development and drying, the layer was sprayed with fluorescamine. Only one amino acid was detected under ultraviolet light (360 nm) and it had the Rf of cystine. The spot was scraped and transferred into a scintillation vial containing 15 ml of Scintiverse I cocktail (Fisher Scientific) for liquid scintillation counting of ^{14}C activity.

Recovery of added ^{14}C in the single amino acid was complete.

3.2.3 Protocol of experiments

Duplicate one gram (oven dry basis-ODB) samples were preincubated at 75% field capacity during 14 days at 20 C. Soil samples were placed in centrifuge cells (Chapter 2) and a dose of ^{14}C-cystine solution was added at time zero to bring the soil water potential to -33 kPa.

3.2.3.1 ^{14}C remaining in solution

Soil solutions were obtained by high speed centrifugation according to Chapter 2. The times for sample collection were : 0, 1, 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 minutes of incubation. Soil samples were sacrificed after each time of solution collection. Soil solutions, usually less than 100 ul, were transferred into scintillation vials containing 15 ml of Scintiverse I cocktail. The ^{14}C activity in the sample was determined after dark adaptation by liquid scintillation counting. A standard sample of known activity was run with all samples. Efficiency of counting was calculated using the sample channels ratio method.

For sterilization experiments, soil samples were autoclaved at 104 kPa for 30 minutes at 124°C. Plate counting of bacteria and fungi followed Wollum (1982).

3.2.3.2 $^{14}CO_2$ measurements

These measurements were made on separate soil samples. A known dose of ^{14}C-cystine was added to a 2 ml autoanalyzer sample cup (Fisher Scientific) containing 1 gram (ODB) of preincubated soil. The cup was then placed in a 20 ml scintillation vial containing 1 ml of 0.2 M NaOH. The $^{14}CO_2$ evolved was collected during 0, 5, 15, 30, 60, 90, 120, 180, 240, and 360 minutes of incubation. After CO_2 collection, the sample cup containing the soil sample was withdrawn and 15 ml Scintiverse I were added to the scintillation vial. The ^{14}C activity was determined as for the soil solution after the fluid in the scintillation vial was clear and homogeneous. Subsequent acidification of the soil indicated CO_2 adsorption by the NaOH was complete.

3.2.4 The models

The premise for the models to be examined is that the simplest model which describes experimental observations is most desirable. More complex representations are used only when simpler ones fail. The simplest representation consists of a microbial and an abiotic component, the latter comprising solid and liquid phases. Pertinent components may be made explicit as:

1. Adsorbed component, consisting of a matrix of electrically charged organo-mineral and microbial cell wall surfaces with adsorptive-desorptive capacities for amino acids (Talibudeen, 1954).
2. Active soil microbial biomass which uses cystine to generate energy and to build essential macromolecules or C skeletons (Anraku, 1980).
3. Soil solution, which contains free amino acids (Chapter 2) and is both a source of readily available nutrients and a sink for plant and microbial metabolites.

These components may be completely or incompletely interconnected producing several cycling patterns (Figure 3-1). At the microsite level, cystine can be released into the soil solution through cell lysis, protease activity, root exudation, metabolic excretion or leakage from cytoplasm of soil microbes. Once cystine molecules are released from organisms or humus, they may be subject to the following basic reactions: i) electrostatic adsorption onto colloidal and microbial cell wall surfaces (Harter, 1977; Gabler, 1978); ii) active microbial uptake to satisfy the cell nutritional and energy needs (Anraku, 1980); and, iii) concurrent desorption from electrically charged surfaces (Paul and Schmidt, 1960).

The model represented by Fig 3-1A is the simplest version. It comprises three basic soil components with substrate transferred among them. The components are interconnected, and each component is kinetically homogeneous.

Model 3-1B permits greater microbially derived diversity in cystine kinetics. Such a model may reflect differing rates of turnover of various groups of microbial constituents such as cytoplasmic or structural components. Cystine can be reduced by enzymatic reactions to cysteine inside microbial cells. Cystine and cysteine are transported into microbial cells where they accumulate in a free state in the cytoplasm. Accumulation values for different aminoacids range between 1.2 and 68 μmoles per 100 mg cells (Holden, 1962). The free cytoplasmic cysteine is used in catabolic and biosynthetic coupled reactions. Cysteine is decomposed to pyruvate by at least three pathways (Meister, 1965). Cysteine can also be incorporated intact into proteins by *de novo* synthesis in *Escherichia coli* (Beilan et al., 1983). On average, the protein content in microorganisms is 60% of the dry cell weight (Mandelstam and McQuillen, 1973). While the above view considers a physiological[5] basis for differing kinetics, a taxonomic (e.g. prokaryotes and eukaryote) basis may be equally valid.

Model 3-1C places greater emphasis on abiotic moieties. Stabilization processes remove substrate from circulation during the time frame of the experiment. Humification, mineral complexation, and physical entrapment of organics may all contribute to stabilization.

The present models assume that reactions transfering substrate-C among soil components follow first order kinetics. Transfer of carbon between abiotic and biontic pools can be described by simple or multiexponential equations. The equations can in theory be used to estimate kinetic parameters such as adsorption, desorption, microbial uptake and oxidation rates, taking place *in vivo* soil systems. On the other hand, relations such as the Monod and Michaelis-Menten models have been used to estimate kinetic parameters for microbially mediated processes (Robinson and Tiedje, 1983). Monod (1942) applied kinetic theory to microbial growth. Parameters of the latter model such as μmax (maximum growth rate) and Ks (substrate concentration at half μmax) are analogous to Michaelis-Menten parameters of Vmax (maximum rate of substrate uptake) and Km (substrate concentration at half Vmax). The rate constant for microbial uptake (k_3)in the models presented in this thesis may be different than Km or Ks values. The value of Ks relates to cell growth and Km to substrate uptake by microbial cells. In comparison, k_3 may reflect microbial growth and/or substrate uptake. The model does not have enough sensitivity to establish a relation between k_3 and Ks or Km.

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[5] In this thesis the physiological term relates to use of a labelled substrate by the soil biontic component for metabolic purposes

Reaction k_3 is treated as first order. That implies that substrate concentration at the enzyme surface is less than Km or Ks, such that the rate of reaction is limited by substrate concentration. Studies of substrate decomposition in soils have used organic substrates added at rates of mg g^{-1} (Sorensen and Paul, 1971; van Veen et al., 1985). The reaction rates under such conditions were also described by first order kinetics, although substrate concentration was much greater than in this study.

The thesis experimental conditions involve the use of ^{14}C-cystine added at quantities of ng g^{-1} soil. This substrate concentration plus adsorption of cystine onto electrically charged surfaces would create conditions of substrate limitation for reactions involving microbial uptake and growth. In addition, Km and Ks values derived from pure culture studies, are based on stirred solutions in which solution concentration would be expected to more closely approximate concentrations at the active site. Such homogeneous conditions do not exist in soil systems. Substrate availability for microbial uptake and growth are conditioned by adsorption and desorption reactions plus the micro-architecture of soils. These abiotic factors can affect apparent Ks and Km. Hence, soils may be properly described by first order reactions such as k_3.

3.2.5 Model selection

The ^{14}C remaining in solution plus the experimental $^{14}CO_2$ values were compared to those simulated by each model to determine if cystine cycling could be described as a 3 (Figure 3-1A) or 4 (Figure 3-1B or 3-1C) component system. If experimental and simulated values were not different (x^2 for goodness of fit), the model was accepted. If they were different, alternate hypotheses were tested.

3.2.6 Numerical analysis for model selection

The following two steps were used to analyze the experimental data: Model 3-1A was examined first.

1. The ^{14}C activity remaining in the soil solution was described as a three component exponential function using BMDP3R (Jenrich, 1983). The program calculates intercepts (H's) and slopes (g's) that define the function. For example, for the soil solution (pool a) the function takes the form:

$$\frac{q_s}{q_{so}} = H_1e^{-g_1t} + H_2\,e^{-g_2t} + H_3e^{-g_3t}$$

where q_s = quantity of tracer in soil solution at time t

q_{so} = quantity of tracer in soil solution at time t = 0.

The amount of ^{14}C in the other pools can also be described by three exponential functions having different intercept coefficients (K's, L's) for compartments b, and c if they exist, but the same slopes (g's) for each component (Shipley and Clark, 1972). Rate constants (k) and the coefficients of intercept (K's, L's) were calculated as described in Chapter 3 and Appendix II of Shipley and Clark, (1972). The product of k times the amount of ^{14}C existing in the microbial pool at different times was integrated to obtain a predicted cumulative $^{14}CO_2$. The latter was subsequently compared to experimental $^{14}CO_2$ measurements.

2. A complete analytical solution through kinetic compartment analysis was not possible when a given hypothesis did not have a completely interchangeable set of reactions between all pools, such as in Figure 3-1B or 3-1C. A non-linear regression model, BMDPAR (Ralston, 1983), provided initial estimates for rate constant (k) values for these cases. This procedure uses a number of differential equations to describe the transfer of labelled substrate-C between pools.

For model 3-1B, the rate of ^{14}C transfer from each pool is defined by the following differential equations:

Soil solution: $\frac{dq_s}{dt} = k_2q_a - q_s(k_1 + k_3)$

Adsorbed : $\frac{dq_a}{dt} = k_1q_s - k_2q_a$

Microbial-1: $\frac{dq_{m1}}{dt} = k_3q_s - q_{m1}(k_4 + k_5)$

Microbial-2: $\frac{dq_{m2}}{dt} = k_4q_{m1} - k_6q_{m2}$

where q_a = quantity of adsorbed tracer

q_{m1} = quantity of tracer in microbial-1

q_{m2} = quantity of tracer in microbial-2.

A three stage process was utilized to estimate rate constant (k) values:

a. Rate constants were varied in a systematic iterative manner until the set of k values predicted the experimental curve for ^{14}C remaining in solution over time.

b. The set of k values selected in "a", was used to simulate the experimental $^{14}CO_2$ measurements. The set of k values was accepted if the simulation did not differ (x^2 for goodness of fit) from the experimental $^{14}CO_2$ curve.

c. If the simulated output differed from the experimental $^{14}CO_2$ curve (x^2 for goodness of fit), then the process returned to "a" and the most sensitive k's were varied until a new set of k's satisfied the experimental curves for both $^{14}CO_2$ and ^{14}C remaining in solution. A given model configuration was rejected if no set of k values simulated the ^{14}C remaining in solution and evolved as $^{14}CO_2$.

Simulation of ^{14}C flow through various compartments was made using the estimated k values together with Continuous system modelling program (CSMP) (Speckhart and Green, 1976).

3.2.7 Flow rates and pool size for unlabelled cystine

The k values and the quantity of soil solution unlabelled cyctine-C (Qs) were used to determine flow rates F(ng min^{-1}) and the quantity (ng g^{-1}) of unlabelled cystine in the adsorbed (Qa), microbial-1 (Qm1), and microbial-2 (Qm2) pools. For the Gray Luvisolic soil, Qs was 111 ng cystine-C (Chapter 2).

Model 3-1B is given as an example. Steady state conditions are required, in which case input flows (F_1) equal output flows (F_2). The quantity of adsorbed cystine was calculated by equating F_1 and F_2 such that $k_1\ Qs = k_2\ Qa$, which is rearranged to $Qa = \frac{k_1}{k_2} Qs$.

Similarly for microbial-1 pool, $Qm1 = \frac{k_3}{k_4 + k_5} Qs$.

Finally for microbial-2 pool, $Qm2 = \frac{k_4}{k_6} Qm1$.

Flow rates (F, ng min^{-1}) were calculated as the product of the respective k value (min^{-1}) times the quantity of cystine-C (ng) in the source of the flow.

3.3 Results

3.3.1 Experimental

3.3.1.1 ^{14}C remaining in solution

The disappearance of ^{14}C from the soil solution and evolved as CO_2 in the Gray Luvisolic and Andept soil are shown in Figure 3-2. The curves showing the ^{14}C activity remaining in solution contain three discernible regions. The first is within the first minute of reaction, where a steep slope is observed. The ^{14}C activity remaining after 1 minute is < 2% of the added dose. A less steep segment was observed in the interval between 1 and 30 minutes. After 60 minutes, the curves changed to a final more horizontal component.

Extrapolation of the tail of each curve to the Y axis shows the ^{14}C-activity to be .06 and .01% of the initial dose in the Gray Luvisolic and Andept samples, respectively. These values represent the equilibrium concentration of ^{14}C-cystine in solution in the presence of living organisms.

The Luvisolic soil was autoclaved to determine if living organisms affected the equilibrium. Over 99% of the colony forming units of both bacteria and fungi were eliminated by autoclaving. Following autoclaving, ^{14}C loss from soil solution was completed within the first minutes of reaction (Figure 3-3). The equilibrium value was nearly two orders of magnitude higher than observed in the presence of living organisms. The subsequent constant concentration of cystine (7.4% of the added dose) therefore represents the equilibrium concentration in the absence of active microorganisms, and may be attributed to independent adsorptive effects.

3.3.1.2 ^{14}C evolved as CO_2

After the addition of labelled substrate, the Gray Luvisolic soil evolved ^{14}C as CO_2 more rapidly than did the volcanic ash derived soil (Figure 3-2). Between 1 and 4% of the initially added substrate-C was evolved during the first 15 minutes of reaction in the Andept and Gray Luvisolic soil, respectively. The rate of CO_2 evolution declined after 40 minutes and started to level off after 240 minutes. The total $^{14}CO_2$ was equivalent to 23% and 29% of the initial dose in each soil, respectively (Figure 3-2).

3.3.2 Model selection for the Andept soil

Step 1 of numerical analysis described the ^{14}C remaining in solution in the Andept soil as the following three component exponential function:

$$\frac{q_s}{q_{so}} = .9977e^{-10.31t} + .0017e^{-.12t} + .0006e^{-.0009t}$$

where the first, second and third term represent the effects of adsorption, microbial utilization and the "equilibrium" concentration, respectively. The changes in amounts of substrate (as %

of initial dose) in the other pools are given by the equations of Table 3-1. The model of Figure 3-1A, simulated $^{14}CO_2$ evolved during 6 hours (Table 3-2).

Step 2 of numerical analysis tested the same hypothesis and rendered similar results. The ^{14}C remaining in solution and evolved as CO_2 predicted by each method was compared to that determined from experimental measurements. A χ^2 test for goodness of fit found no significant differences between the predictions of either method and the experimental observations (Table 3-2), although there was a trend toward better prediction of CO_2 evolution using step 2. The data appear to be insensitive to the method of calculation for this soil; suggesting that for completely interconnected systems either method is satisfactory. Predicted k values estimated by both methods were also not different (Table 3-3).

Models 3-1B and 3-1C were also examined by step 2 of numerical analysis. Output for 3-1B rendered k_4 and $k_6 = 0$, suggesting that the experimental data for the Andept soil cannot be explained by such a model. In contrast, output from model 3-1C closely simulated the ^{14}C remaining in solution and evolved as CO_2 (Table 3-2). Consequently, it is not possible to distinguish clearly between model 3-1A and model 3-1C for this soil.

3.3.3 Model selection for the Gray Luvisolic soil

The dynamics of ^{14}C cystine in the Gray Luvisolic soil was not described by the three compartment model of Figure 3-1A nor by the four component model of Figure 3-1C. The former model simulated the ^{14}C evolved as CO_2 only over the first 120 minutes of reaction. Beyond this time the model overestimated $^{14}CO_2$ evolution such that by 360 minutes the predicted CO_2 was about twice the measured quantity and the curve had not levelled off. Some stabilization process appeared to be operating to cause evolved CO_2 to level off after 40 minutes. Hypothesis of model 3-1B involves a second microbial component that stabilizes some of the organic substrate. Model 3-1B closely predicted the ^{14}C remaining in solution and evolved as CO_2 beyond 5 minutes (Figure 3-4), while accounting for the levelling off in CO_2 evolution rate after 40 minutes. Model 3-1C (abiontic stabilization) generates a linear cumulative CO_2 evolution trend, and did not account for the change in respiration rate after 40 minutes.

3.3.4 Rate constants

For both soils, the fastest average cycling pool was the soil solution and turnover rates decreased in the following order: soil solution > microbial-1 > adsorbed > microbial-2 (Table 3-4). Estimated microbial uptake rate (k_3) is smaller than the adsorption rate (k_1) in both soils when 4 component models are used. Low desorption relative to microbial uptake rates combine to keep solution concentrations low. Respiration rates were among the slower specific rates in both soils regardless of the model or calculation method.

A simple sensitivity analysis was made to study the model behaviour. Each rate constant was changed by 50% from that estimated by numerical analysis to define the model parts which are most sensitive.

The ^{14}C activity remaining in the Luvisolic soil solution was more sensitive to changes in adsorption (k_1) and microbial uptake (k_3) than to desorption (k_2) rates. Conversely, CO_2 evolution was affected mainly by uptake (k_3), adsorption (k_1) and oxidation rates from both microbial pools (k_5 and k_6).

For the Andept soil system represented by Figure 3-1C, the ^{14}C remaining in solution is most sensitive to uptake (k_3) and to stabilization (k_7) rates. The processes of adsorption (k_1), microbial uptake (k_3) and respiration (k_6) were the most important controls on CO_2 evolved from the volcanic soil.

3.4 Discussion

Whereas the literature deals with bulk soil measurements, the present study treats specific compartments and the major processes and agents affecting them. The present data were generated over short intervals and for a short incubation time. Extrapolation of the models to longer times is not valid because slower processes which are not expressed over short periods may have profound effects on the long term fate of cystine in bulk soils. These models are, however, valid for the rapid dynamics characteristic of soil solutions. The work reported here deals with ^{14}C-labelled cystine added to soil and with subsequent dynamics of ^{14}C originating from that substrate. Reference to cystine is used to indicate a material that behaves kinetically in the same way as added labelled cystine; it should not be construed as isolated and purified cystine.

The present data show a rapid turnover rate of labelled cystine through the soil solution. This is consistent both with the view that in natural soil systems the inhabitant microflora are under conditions of starvation; and with pure culture studies which have shown that under nutritional deficiencies, there is a greater demand for amino acids. Specific transport systems for L-cystine and L-methionin are derepressed in ***Penicillum*** under sulphur starvation (Skye and Segel, 1970; Benko *et al.*, 1967). Starvation for either carbon or nitrogen derepresses a general non-specific amino acid transport system for neutral and basic amino acids (Benko *et al.* 1967). However, *Agrobacterium radiobacter* failed to utilize cysteine from a Montmorillonite-, Kaolinite- amino acid system (Dashman and Stotsky, 1986).

Longer turnover times were observed for the microbial components with almost two orders of magnitude difference between fast and slow microbial constituents. Only 20 - 30% of the added ^{14}C was evolved during 6 hours, and continued stabilization of the remainder as either microbial or physico-chemical complexes in soil is indicated. Such rapid rates are obvious only when short intervals are sampled. Consequently sampling over 24 hour periods (Sorensen and Paul, 1971) yield longer estimates because they integrate the data over longer periods. The data of Sorensen and Paul (1971) are, however, consistent with the tendency observed here for the model outputs to simulate accumulation of cystine-C in progressively less active components.

Adsorption and desorption are major processes in the models, but they are not treated using classical isotherms. In soil systems, descriptions and interpretation of adsorption processes use the Freundlich, Langmuir and Tempkin isotherms (Mead, 1981). These isotherms provide values for constants of equilibrium, and maximum capacity of adsorption, but do not define the kinetics of concurrent adsorption-desorption reactions. Also, the common curve fit test for linearity of the Langmuir plot has been critized by Harter (1984), who states that at low substrate concentrations, the equation can provide 50% error in the estimate of adsorption maxima. In soils, adsorption reaction rates may decline in progressing from the beginning of the reaction towards the end of the process (Van Riemsdijk and Van der Linden, 1984). Amacher *et al.* (1986) concluded a multi-component model comprising both reversible and irreversible phenomena was needed to describe retention and release of metals by soils, with irreversible processes increasing in importance as time progressed. Further, classical experimental methodology to obtain such isotherms is based on batch techniques, whereby soils and a solution of known composition and concentration are shaken between 1 and 72 hours to attain chemical equilibrium (Singh, 1984). Mechanical disturbances such as shaking, collection of supernatant solutions after filtration (Barrow and Shaw, 1979), and the reaction time (Zasosky and Burau, 1978), often make interpretation uncertain (Van Riemsdijk and Van der Linden, 1984).

In nature, none of the latter conditions exist for soil systems. The above methods ignore effects of soil microorganisms on chemical equilibria of soil solutions. While in the present studies the initial process must be attributed mainly to adsorption of cystine by an abiotic component, the equilibrium obtained after the first ten minutes reflects a biontic influence. Soil microbes irreversibly withdraw substrate from the solution thereby continuously altering the "chemical equilibrium" between solution cystine and the adsorbed pool. Boyd and King, (1984) proposed an anaerobic batch technique to determine adsorption of rapidly degradable substrates. Although this method eliminates some problems, it cannot account for anaerobic microbial processes. Kinetic analysis, however, treats both abiotic and biontic contributions explicitly and simultaneously. The models define active microbial biomass as an important agent stabilizing and controlling the cycling of free cystine through the solution phase of these two soils. This control is regulated by internal metabolic processes which in turn are related to the soil solution composition (Bosatta and Agren, 1985), type of microbial populations, and environmental factors which stress microbial activities.

The Andept soil can be described by either a 3 or 4 component model with an abiontic stabilization process. Interpretations from the kinetic model together with information already published for volcanic soils (Aomine and Kobayashi, 1964; Borie and Zunino, 1983) strongly suggest the concept of one biontic and one stabilized component (model 3-1C) rather than the leakage of ^{14}C metabolites from microbial cells into solution (model 3-1A). If model 3-1C is accepted, the dynamics of cystine would be described by Table 3-5a. For both models of the Andept, an important proportion of ^{14}C is transferred into one microbial component which is kinetically similar to microbial-2 pool of the Luvisolic soil. The quantity of microbial ^{14}C would equal the quantity adsorbed after 360 minutes (Table 3-5a). Stabilized ^{14}C would dominate over longer times. The model predictions complement the findings that newly formed biomass-C turns over very slowly in this soil (Monreal and McGill, 1981) and helps in part to explain the net organic matter accumulation in such soils.

A four component model having a second stable biontic component describes short term cystine kinetics in the Luvisolic soil. In this soil, the microbial-2 component is the major sink after 6 hours (Table 3-5b) and is reflected in calculations of steady state pool sizes (Table 3-6). In the Gray Luvisolic soil the flows of cystine-C into and from the adsorbed pool were equivalent at 233 ng C min^{-1}. The soil solution was the smallest pool with 111 ng C; the largest were the adsorbed and the microbial-2 pools containing 25900 and 73920 ng C, respectively. The desorption of 233 ng C min^{-1} would indicate that adsorption only retards the substrate utilization by the soil biota. A flow of 100 ng min^{-1} cystine-C into the system defined by the model will maintain the steady-state condition in the Gray Luvisolic soil. Such a rate could not be maintained in soil without substantial C input from plants. The potential for depletion of soil organic reserves is shown by these data to exceed likely inputs. If such ^{14}C distributions hold for a wide range of soils, they would suggest a major involvement of biomass in organic matter stabilization. The 4.8 y turnover time for amino acids formed in situ after soils were pretreated with ^{14}C cellulose (Sorensen, 1975) is consistent with such stabilization. These models are also consistent with concepts proposed by Van Veen *et al.* (1985) to account for stabilization of organic materials in Australian soils.

In conclusion, flows of ^{14}C-cystine through the solution of two soils have been described by using kinetic analysis and concepts of kinetic homogeneity. Two separate mathematical approaches were equally successful in describing the data for the Andept soil which could be described as either completely or incompletely interconnected. The Gray Luvisolic soil could be described only as an incompletely interconnected system.

The models are consistent with abiotic and biontic stabilization mechanisms for the Andept soil and biontic stabilization of cystine-^{14}C in the Luvisolic soil. Turnover times vary from seconds for the solution pool to hours for microbial cystine during the first six hours. Turnover time of stabilized cystine ^{14}C in the Andept was too long to be measured using the present incubation times. The microbial components were the major sinks for cystine in the Luvisol; whereas the colloidal stabilized and a microbial pool became the major sink in the Andept.

Kinetically distinguishable phases or compartments of the soil system have been separated. The boundaries of these pools may not conform to anatomical or chemical entities. The identities of the microbial-1 and microbial-2 components have yet to be physiologically or chemically defined.

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Table 3-1. Functions describing the dynamics of ^{14}C-cystine through an Andept soil using the three compartment model described by Figure 3-1A

Soil	Pool	$A_1e^{-g_1t}$	+	$A_2e^{-g_2t}$	+	$A_3e^{-g_3t}$
Andept	Solution	$.9977e^{-10.31t}$	+	$.0017e^{-.12t}$	+	$.0006e^{-.0009t}$
	Adsorbed	$-.1415e^{-10.31t}$	+	$.1351e^{-.12t}$	+	$.0064e^{-.0009t}$
	Microbial	$-.8562e^{-10.31t}$	—	$.1380e^{-.12t}$	+	$.9942e^{-.0009t}$

$A_{1,2,3}$ = Represent the intercept coefficients for the solution (H's), adsorbed (K's) and microbial (L's) pools.

Table 3-2. Comparison of two numerical methods and two models to describe the cycling of ^{14}C-cystine in an Andept soil under laboratory conditions.

	^{14}C (as % of initial dose)							
	CO_2				Soil Solution			
		Predicted				Predicted		
Time (min)	Measured	Step 1 3 pools[1]	Step 2 3 pools	Step 2 4 pools[2]	Measured	Step 1 3 pools	Step 2 3 pools	Step 2 4 pools
0	0.00	0.00	0.00	0.00	100.0000	100.0000	100.0000	0.0000
5	0.37	0.37	0.35	0.28	0.1558	0.1514	0.1756	0.0973
17	1.23	1.40	1.29	1.00	0.0816	0.0779	0.0903	0.0708
30	2.62	2.17	2.33	1.80	0.0616	0.0584	0.0675	0.0683
60	5.22	4.33	4.75	3.71	0.0475	0.0518	0.0602	0.0627
90	7.75	6.44	7.10	5.69	0.0441	0.0502	0.0571	0.0577
120	10.21	8.50	9.40	8.11	0.0416	0.0489	0.0561	0.0531
180	14.94	12.44	13.83	12.20	0.0400	0.0463	0.0517	0.0449
240	19.42	17.18	19.03	17.21	0.0366	0.0439	0.0504	0.0379
360	23.54	23.07	24.24	24.00	0.0333	0.0394	0.0428	0.0291
x^2		0.71	0.27	1.04		0.03	0.12	0.25

x^2 = 3.84 at $p = .05$ with 1 degree of freedom.

[1] The three pool system corresponds to Figure 1a.

[2] The four pool system corresponds to Figure 1c.

Table 3-3. Comparison of rate constant (k) values estimated by two numerical methods for a three compartment model describing the dynamics of ^{14}C-cystine through an Andept soil.

Predicted reaction		Step 1	Step 2
Adsorption	(k_1)	1.40	1.95
Desorption	(k_2)	0.13	0.13
Microbial uptake	(k_3)	8.80	8.98
Excretion	(k_4)	0.005	0.005
Respiration	(k_6)	0.001	0.0009

Table 3-4. Predicted rate constant (k, min^{-1}) values for reactions affecting the cycling of ^{14}C cystine in two soils.

Soil	Solution (ks)	Microbial-1 (kml)	Adsorbed (ka)	Microbial-2 (km2)	k_1†	k_2	k_3	k_4	k_5	k_6	k_7	k_8
Gray Luvisolic	3.0	.66	.009	.0010	2.1	.009	.90	.48	.17	.0010		
Andept (Model 1c)	8.4		.009	.0025	6.5	.008	1.87			.0025	.001	
Andept (Model 1a)	10.2		.130	.0060	1.4	.13	8.8			.0010		.005

†k values represent the rates of k_1 = adsorption, k_2 = desorption, k_3 = microbial uptake, k_4 = internal microbial transfer, k_5 = respiration from microbial-1, k_6 = respiration from microbial-2, k_7 = stabilization and k_8 = excretion.

Table 3-5a. Dynamics of ^{14}C-cystine in Andept soil samples defined as the four compartment model of Figure 3-1C.

Time (min)	^{14}C in compartment (% of initial dose)			
	Solution	Adsorbed	Stabilized	Microbial
0	100.00	0.0	0.0	0.0
5	0.08	76.5	0.4	22.7
30	0.07	71.4	2.2	24.5
60	0.06	65.7	4.3	26.3
120	0.05	55.6	7.9	28.5
360	0.03	28.2	17.6	28.6

Table 3-5b. Dynamics of ^{14}C-cystine in Gray Luvisolic soil samples defined as the four compartment model of Figure 3-1B.

Time (min)	^{14}C in compartment (% of initial dose)			
	Solution	Adsorbed	Microbial-1	Microbial-2
0	100.00	0.0	0.00	0.0
5	0.21	69.0	1.70	21.4
30	0.18	64.7	0.25	25.1
60	0.17	60.0	0.24	27.8
120	0.15	51.3	0.20	32.4
360	0.08	27.7	0.11	40.9

Table 3-6. Pool size and flow rate values for ^{12}C-cystine cycling through the Gray Luvisolic soil defined as the four compartment model of Figure 3-1B.

Pool size	Solution	Microbial-1	Adsorbed	Microbial-2
Q (ng C g^{-1})	111	154	25900	73920

Flow rate	Adsorbed	Desorbed	Uptake	Internal Transfer	Microbial-1 Respiration	Microbial-2 Respiration
F(ng C g^{-1} min^{-1})	233	233	100	74	26	74

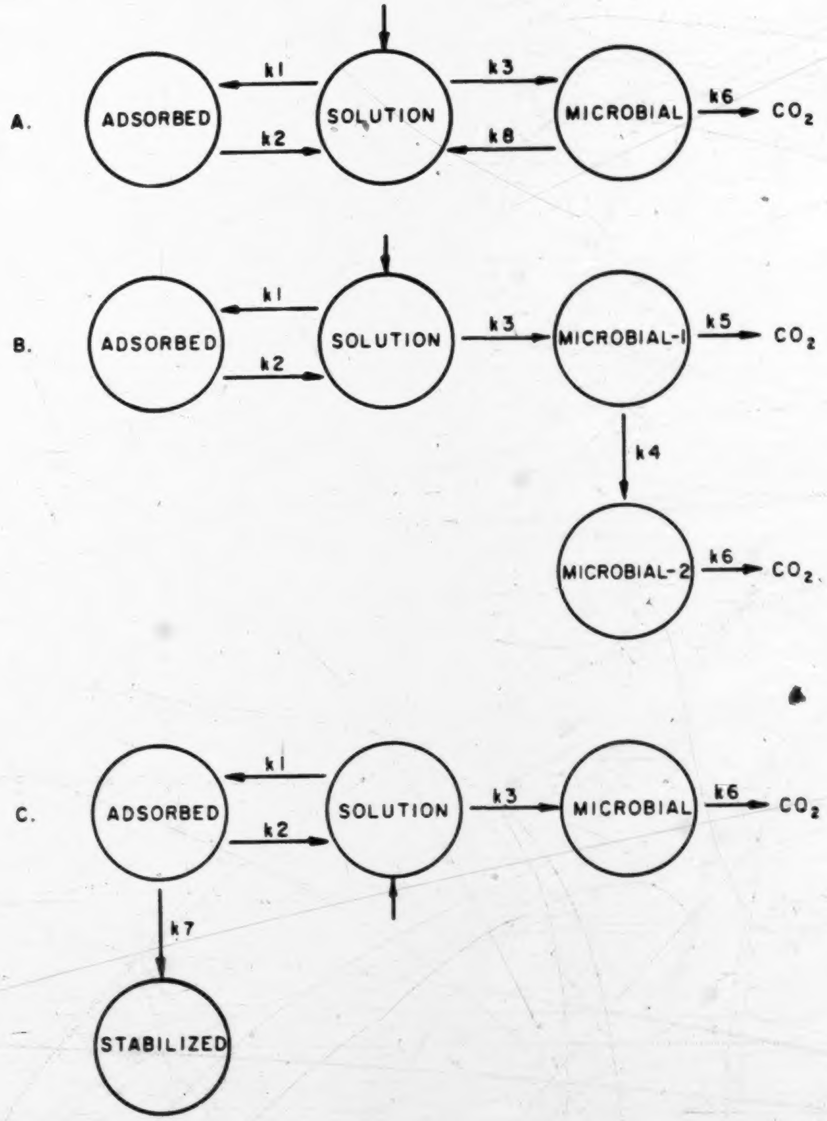
A.
ADSORBED
SOLUTION
MICROBIAL
k1
k2
k3
k8
k6
CO2
B.
ADSORBED
SOLUTION
MICROBIAL-1
MICROBIAL-2
k1
k2
k3
k5
k4
k6
CO2
CO2
C.
ADSORBED
SOLUTION
MICROBIAL
STABILIZED
k1
k2
k3
k6
k7
CO2

Figure 3-1. Hypotheses representing the cycling of free cystine through a three and four compartment soil ecosystem.

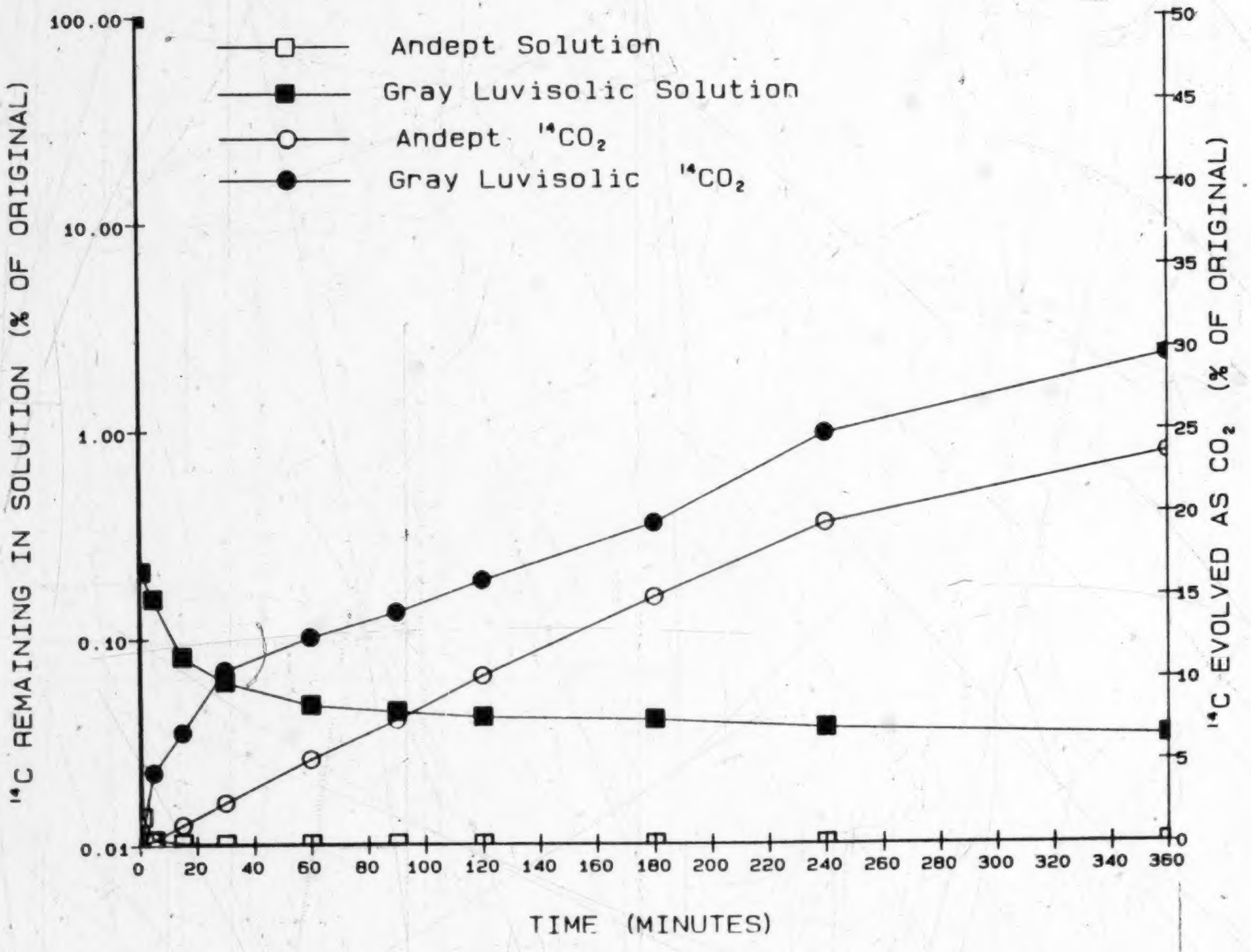

Figure 3-2. The ^{14}C remaining in solution and evolved as CO_2 in a Gray Luvisolic and an Andept soil.

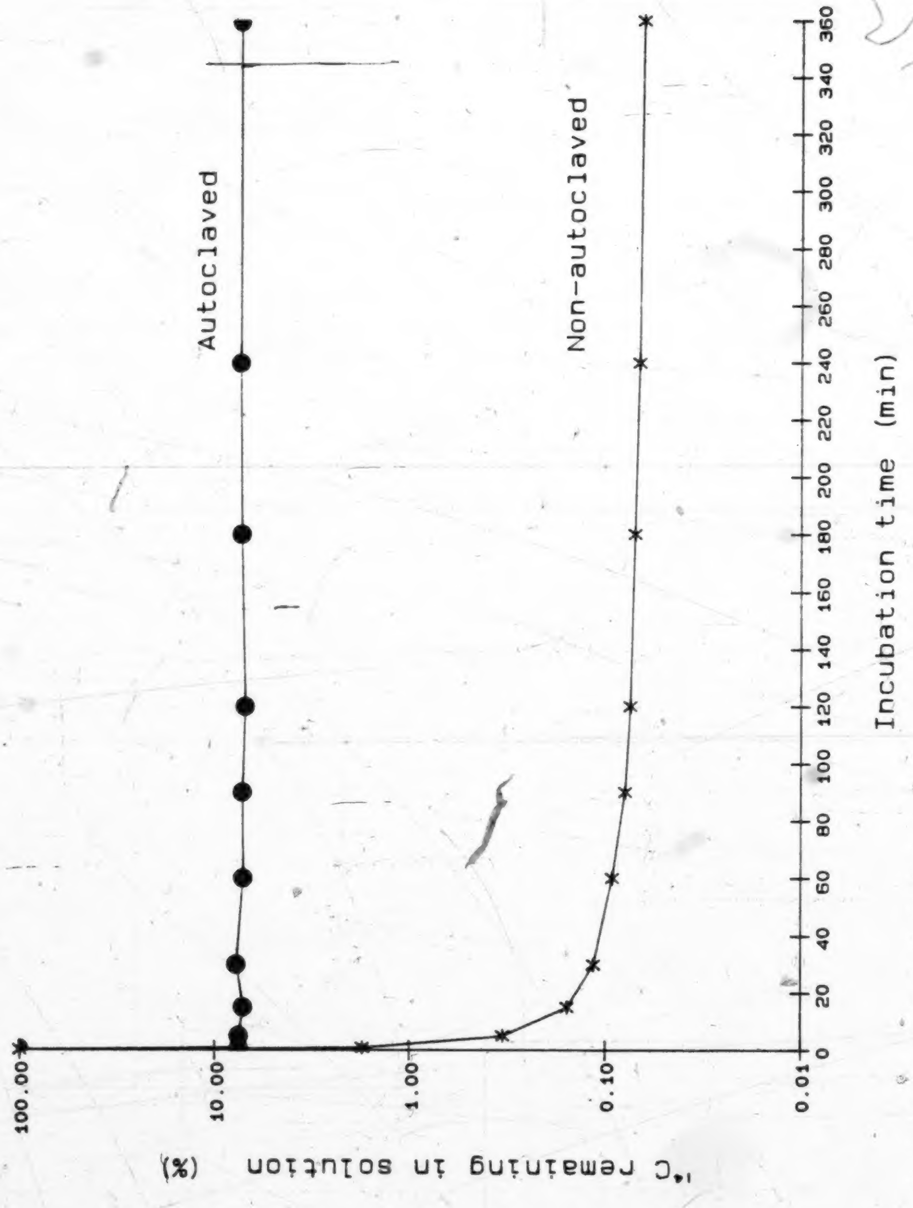

Figure 3-3. The ^{14}C activity remaining in the solution of autoclaved and non-autoclaved samples of a Gray Luvisolic soil.

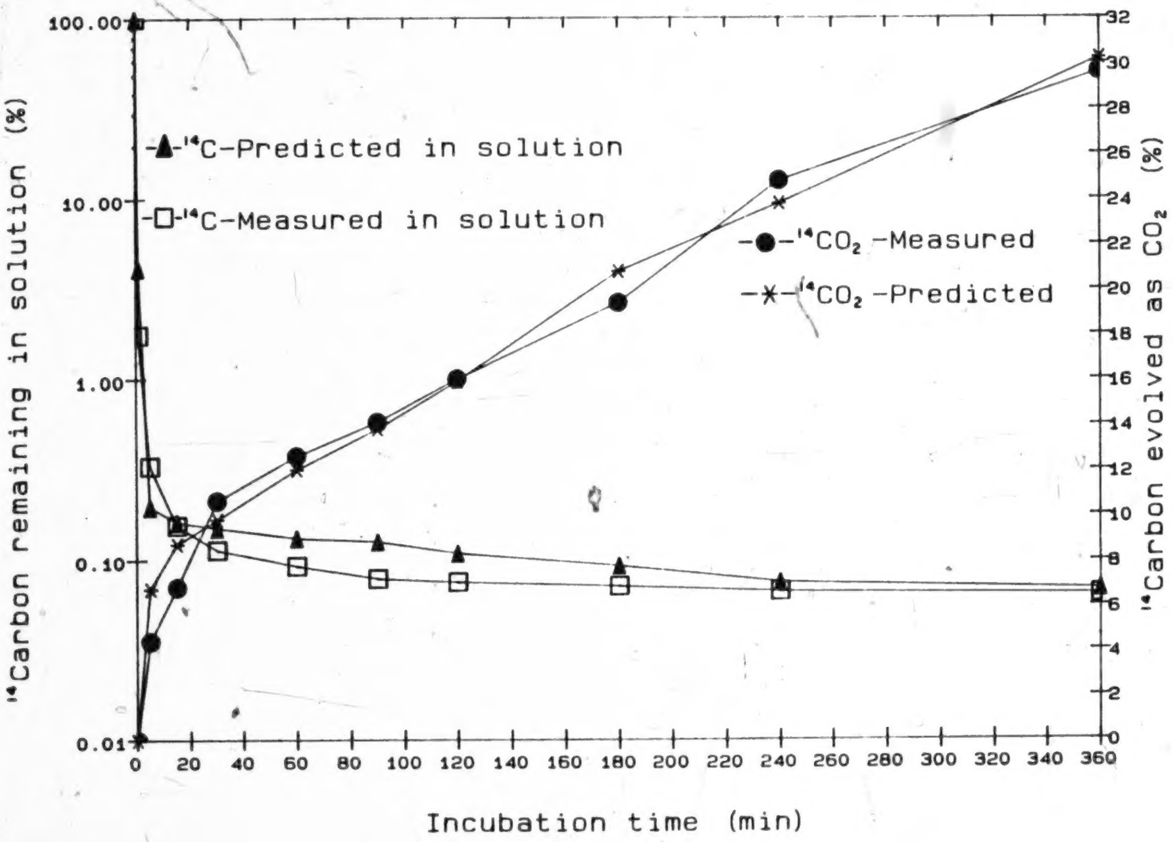

Figure 3-4. The measured and predicted ^{14}C activity remaining in solution and evolved as CO_2 in a Gray Luvisolic soil described as the 4 compartment model of Figure 3-1b.

4. KINETIC ANALYSIS OF MICROBIAL FREE AMINO ACID AND CELL PROTEIN POOLS IN A GRAY LUVISOLIC SOIL[1]

4.1 Introduction

Knowledge about the kinetics of internal cycling processes can assist in understanding the extent to which various control mechanisms affect energy flows and nutrient availability to plants. The kinetics of free cystine cycling through soil solutions of a Gray Luvisolic soil at steady-state was described in Chapter 3. The kinetic model suggests four compartments affect the flow of substrate through this soil. The pools are the soil solution, adsorbed phase, plus two microbial components that control the internal cycling of cystine. The latter two pools were not characterized in Chapter 3.

Perturbation of soil systems frequently results in growth and/or death of soil microorganisms, with altered kinetics of nutrient flow. In perturbed soil systems the utilization of excess glucose (1000 $\mu g\ g^{-1}$ soil) has been attributed primarily to combined bacterial and fungal respiration (Anderson and Domsch, 1975). In aquatic environments, however, bacteria constitute the main group of microorganisms that rapidly utilize dissolved organic substrates (Hobbie 1973; Ferguson and Sundra, 1984). Protein turnover rates vary between growing and stationary cells (Mandelstam, 1963) and are closely regulated (Bachmair *et al.* 1986). Comparisons of soil systems perturbed by added excess glucose with non-perturbed systems provide a test of model sensitivity to physiological conditions of soil microbial biomass. They can also provide insights to the identity of the two microbial pools kinetically defined in Chapter 3. Further insights into the identity of those two components may be obtained from comparison of model outputs with relative bacterial and fungal contributions to respiration as proposed by Anderson and Domsch (1975).

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[1] A version of this chapter will be submitted for publication. C.M. Monreal and W.B. McGill (Soil Biology and Biochemistry).

The objectives of these studies were to:

a) model the cycling of excess glucose-C under non steady-state condition; and

b) extend the earlier kinetic study with cystine (Chapter 3) to discover if the two kinetically defined microbial pools can be associated either with different microbial populations or with internal cell components.

Two alternative hypotheses are proposed to assist in meeting these objectives:

i) cystine cycles mainly through two taxons: prokaryotes and eukaryotes who use the substrate to generate energy and/or carbon skeletons and as a source of nitrogen and sulfur to satisfy some of their metabolic needs.

ii) the microbial components represent internal cell pools that serve to store, translocate and/or transform the substrate to satisfy cellular metabolic needs.

4.2 Materials and methods

4.2.1 Soils

Samples from a Gray Luvisolic under a 2 year crop-fallow rotation and from a Dark Brown Chernozemic virgin soil were used for experimental purposes. The latter soil was used in a study to determine bacterial and fungal contributions to cystine respiration. Chemical and physical properties of both soils are described in Chapter 2.

4.2.2 Protocol of experiments

Incubation studies using ^{14}C-cystine as the substrate and the experimental measurements of ^{14}C remaining in solution and evolved as CO_2 were made as described in Chapter 3. Glucose was added at a rate of 1000 μg g^{-1} soil. The specific activity of ^{14}C glucose was 519 Bq mg^{-1} of substrate.

4.2.3 Bacterial and fungal respiration

The proportions of the total CO_2 contributed by bacterial and fungal populations were estimated following the antibiotic method of Anderson and Domsch (1975).

4.2.4 Statistics

The incubation studies were treated as simple factorial experiments. Analysis of variance was conducted for the ^{14}C remaining in solution and evolved as CO_2 over time. Soil samples were duplicated for each time of measurement. Duncan's multiple range plus Least significant difference tests were applied to determine statistical differences between treatments (Zar, 1984).

4.2.5 The models

The models examined for the cycling of excess glucose and cystine are based on theoretical considerations. The simplest configuration describing experimental observations was considered the preferred model.

4.2.5.1 Glucose dynamics in a perturbed soil system

Glucose is not subject to adsorption reactions to electrically charged colloidal surfaces. However, molecules trapped between clay layers or in micropores may not be available for microbial use. Substrate molecules are transfered from solution into active soil microbial cells. Four sub-hypotheses were established:

Subhypothesis 1: Labelled glucose is taken up separately by two microbial populations, each having its own uptake (k_1 and k_2) and oxidation (k_3 and k_4) reactions to CO_2 (Figure 4-1A).

Subhypothesis 2: Labelled glucose enters one microbial population and ^{14}C is allocated into intermediates of metabolism (I) and macromolecules (M). Under growing conditions the ^{14}C allocated into (M) has no turnover (Figure 4-1B).

Subhypothesis 3: Similar to subhypothesis 2 with the exception that two microbial populations oxidize ^{14}C from internal cellular pools. For non-stationary growing conditions the rest of the ^{14}C is used to build C skeletons for fast cell growth (k_3,k_4) (Figure 4-1c).

Subhypothesis 4: Labelled glucose cycles through a five compartment system with two microbial populations controlling the dynamics of ^{14}C. Each population has separate uptake reactions (k_1 and k_2). Two internal compartments for each population oxidize ^{14}C to CO_2 (k_3, k_4 and k_7, k_8) (Figure 4-1d). Cycling of glucose-C was also tested for models of Figure 4-2. The adsorbed component would be equivalent to glucose molecules trapped within micropores or the interlayer spacing of clay colloids.

4.2.5.2 Cystine dynamics under non-perturbed conditions

Two main hypotheses were tested to define the cycling of free cystine through a four compartment system having two microbial moieties. The first hypothesis proposes that ^{14}C-cystine cycles through two microbial taxa: prokaryotes and eukaryotes (Figure 4-2A); the second proposes two cellular constituents: cytoplasmic free amino acids and cell proteins (Figure 4-2B). Alternatively, hypotheses of Figure 4-2B may represent ^{14}C allocation into cytoplasmic and cell wall components, rather than cytoplasmic amino acids and proteins. Cycling of cystine-C was also tested for models of Figure 4-1.

4.2.6 Model selection

The experimental $^{14}CO_2$ curve obtained from the glucose treated samples was compared to simulated model outputs to determine if the cycling of excess glucose could be described by models represented by Figures 4-1 and 4-2.

Values for ^{14}C remaining in solution and evolved as CO_2 were compared to simulated outputs to determine if cystine cycling is described by models represented by Figures 4-1 and 4-2.

4.2.7 Numerical analysis for model selection

The numerical steps used BMDPAR and CSMP as described in Chapter 3.

The changes in ^{14}C-glucose for the four subhypotheses described by Figures 4-1 were defined by differential equations.

For example for subhypothesis 1:

$$\frac{dq_s}{dt} = -(k_1 + k_2)q_s;$$

$$\frac{dq_{p1}}{dt} = k_1 q_s - k_3 q_{p1}; \text{ and}$$

$$\frac{dq_{p2}}{dt} = k_2 q_s - k_4 q_{p2}$$

where q_s = amount of ^{14}C in the soil solution

q_{p1} = amount of ^{14}C in population-1

q_{p2} = amount of ^{14}C in population-2

Analogous differential equations were written for the remaining subhypotheses of Figure 4-1 and used notations:

q_i = amount of ^{14}C in pool of intermediates

q_M = amount of ^{14}C as macromolecules

q_{p1i} = amount of ^{14}C as intermediates in population-1

q_{p1M} = amount of ^{14}C as macromolecules in population-1.

The changes in ^{14}C-cystine for the two hypotheses described by Figure 4-2A and 4-2b were defined by the following differential equations:

Hypothesis 1:

$$\frac{dq_s}{dt} = k_2\, q_a - (k_1 + k_3 + k_4)\, q_s$$

$$\frac{dq_a}{dt} = k_1 q_s - k_2 q_a$$

$$\frac{dq_{p1}}{dt} = k_4 q_s - k_6 q_{p1}$$

$$\frac{dq_{p2}}{dt} = k_3 q_s - k_5 q_{p2}$$

where q_s, q_{p1}, q_{p2} were defined previously and

q_a = amount of ^{14}C adsorbed to electrically charged surfaces.

Analogous equations were written for hypothesis 2 with the additional notation:

q_{CYT} = amount of ^{14}C in the cytoplasmic free amino acid pool

q_{PROT} = amount of ^{14}C in cell proteins.

4.2.8 Pool size and flow rates

Calculation of these parameters was made as described in Chapter 3. For the perturbed Luvisolic soil the glucose-C concentration in solution (Qs) was equal to the amounts added during the incubation studies (400 $\mu g\ g^{-1}$ soil). Qs for cystine-C is 111 $ng\ g^{-1}$ soil (Chapter 2).

4.3 Results

4.3.1 Bacterial and fungal respiration of glucose in a perturbed soil system

Samples of a Gray Luvisolic soil were supplemented with ^{14}C-glucose and the antibiotics actidione and streptomycin to estimate bacterial and fungal contributions to soil respiration. Although addition of the inhibitors caused partial losses in microbial activity, close to 70% of the respiration remained in the presence of the combined antibiotics as shown by the area under curve D (Figure 4-3). After 6 hours of incubation only 6% of the added glucose-^{14}C was respired to CO_2. The average bacterial and fungal contributions to respiration were estimated to be 35 and 65%, respectively.

4.3.2 ^{14}C-cystine oxidation in non-perturbed soils

In natural soil systems stationary growth prevails due to nutrient limitation. An attempt was made to use the antibiotic method of Anderson and Domsch (1973) in cystine amended soils. Small amounts of labelled cystine were added to samples to maintain steady-state conditions of the soils. In the control samples 23% of the added cystine-^{14}C was oxidized to CO_2 after six hours. The addition of antibiotics increased respiration of ^{14}C-cystine slightly from 21 to 28% in the Luvisolic soil. A repeat of the same experiment using a Dark Brown soil rendered similar results (Table 4-1)

4.3.3 Model selection for excess glucose-C cycling in a perturbed soil system

The models of Figure 4-1 and 4-2 were tested to describe ^{14}C cycling in a soil supplemented with excess glucose. Model 4-1A and 4-1D underestimated $^{14}CO_2$ evolved during the first 5 hours of reaction. Output from models 4-1B and 4-1C closely predicted the CO_2 evolved (Figure 4-4). The latter two models include a microbial component that stabilizes some of the organic substrate.

4.3.4 Model selection for cystine cycling in non-perturbed soils

Kinetic analysis was extended to examine cystine dynamics under stationary steady-state growing conditions. For hypothesis 1 (Figure 4-2A) predicted ^{14}C remaining in solution of the Gray Luvisolic soil is different from that observed during experimental measurements (Table 4-2). This suggests that cycling mechanisms operating in the soil are not described by such a model.

Hypothesis 2 represented by Figure 4-2B was accepted for cystine-cycling under stationary growing conditions. The output of this model mimics measurements of ^{14}C both evolved as CO_2 and remaining in solution (Table 4-3). Models of Figure 4-1 were also tested.

Every model predicted the experimental CO_2 values but no model matched ^{14}C remaining in solution.

4.3.5 Rate constant and pool size

According to models 4-1B and 4-1C, ^{14}C-glucose allocated into pool (M) is not degraded during the experiment. The size of pool (i) was estimated to be 31 and 52 $\mu g\ g^{-1}$ of C by models 4-1B and 4-1C, respectively. In model 4-1C glucose uptake rate (k_3) is low and the respiration rate (k_7) from population-2 is almost negligible (Table 4-4). Simulated CO_2 evolution from model 4-2B closely mimicked the experimental values, but $k_1 = 8 \times 10^{-4}\ h^{-1}$ and $k_6 = 3 \times 10^{-7}\ h^{-1}$ are insignificant, which suggests that adsorption and respiration from a second microbial component are negligible. Rate constant values for cystine cycling in the Luvisolic soil as defined by model 4-2B were described in Chapter 3.

4.4 Discussion

In the present study, kinetic analysis and modelling have been used to describe the cycling of glucose in perturbed soil systems and that of cystine under non-perturbed conditions. The models for glucose-C cycling were validated by comparing one experimental variable (CO_2) to the model output. Therefore, the validation for glucose is less rigorous than for models of cystine where two experimental variables were incorporated into the model selection process.

4.4.1 Dynamics of ^{14}C-glucose in a disturbed soil system

Respiration of C from an intermediate pool with concurrent generation of a non-respiring moiety is characteristic of both models (4-1B, 4-1C) which describe glucose cycling under perturbation. Model 4-1B is simpler and is consistent both with pure culture studies and with soil level data. From pure culture studies it is known that growing microbial cells use organic substrates for the biosynthesis of low molecular weight organic molecules, macromolecules and provision of energy to achieve the first two activities (Hawker and Linton, 1971). In growing cells, proteins as well as RNA are degraded slowly or not at all (Willets, 1967; Mandelstam, 1963). At the soil level, Coody *et al.* (1986) calculated a balance sheet for glucose-C after various soil fractions were chemically identified. In their studies, uptake rate of glucose-C exceeded its oxidation rate, resulting in accumulation of organic-C within microbial cells. This is consistent with output from model 4-1b. In the present incubation studies, the concentration of added glucose at 25% soil moisture content was equivalent to 20 mM. The rate of respiration was similar to that reported by Coody *et al.* (1986) who incubated soils with a 20 mM glucose solution at 20°C.

The contributions of bacteria and fungi to glucose respiration in the Gray Luvisolic soil was comparable to values reported by Anderson and Domsch (1975) but differed from those reported by Song *et al.* (1986) who attributed 82% of n-hexadecane mineralization in soil to bacterial activity. The kinetic analysis, however, revealed that flows of C from each microbial moiety of model 4-1C did not match the respective 35 and 65% contributions to soil respiration. The experimental data are sensitive to antibiotics which in turn affected only the respiration of some soil microorganisms. On the other hand, the present kinetic model defines components on the basis of rates at which ^{14}C flows through them. Consequently, the model output is sensitive to reaction rates rather than to metabolic inhibitors. Unless sensitivity to antibiotics coincides with relative reaction rates, the antibiotic and kinetic approaches must yield different results. Conversely, a kinetic analysis will separate bacterial and fungal components only if both taxa exhibit different reaction rates. Therefore, it is concluded that controls on glucose-C dynamics under perturbed conditions are more physiological than taxonomic.

The high proportion of $^{14}CO_2$ respired from the antibiotic treated samples can be associated with the following factors:

1. Adsorption of streptomycin to soil colloids renders some molecules inactive (Siminoff and Gottlieb, 1951).

2. Microbial degradation of streptomycin and actidione (Pramer and Starkey, 1951).
3. Metabolism of ^{14}C glucose by microorganism resistant to both antibiotics. Many bacteria are resistant to streptomycin. Plasmids, extra-chromosomal DNA, determine resistance to the antibiotic. In addition to these plasmids, other extracellular factors can contribute to the inactivity of streptomycin (McQuillen, 1973).

4.4.2 Dynamics of ^{14}C-cystine in a non-perturbed soil

The addition of the antibiotics streptomycin and actidione to non-perturbed soils tended to stimulate respiration of ^{14}C-cystine from both soils studied. Earlier reports indicate neutralization or inactivation of streptomycin can be accomplished by anaerobic environments, sulphydryl compounds, H_2S, hydroxylamine, ketone reagents, cevitamic acid, glucose and by cysteine (Waksman, 1947). Inactivation by cysteine occurs via condensation of the aldehyde group of streptomycin with the thiol and amino groups of cysteine which results in the formation of thiozolidine, an inactive product (Korzybski *et al.* 1978). In our experiments, cystine an intermediate of cysteine oxidation may have started a similar reaction. On a molar basis, a potential reaction of cystine with streptomycin cannot account for the drug's inactivation. Thus the greater microbial respiration in the antibiotic treated samples has to be associated with other soil factors which are as yet unknown. Tests of hypotheses of Figure 4-2A and 4-2B used data from the kinetic analysis and evidence provided by the glucose treatments. Results of the kinetic analysis indicated model 4-2a did not represent ^{14}C-cystine dynamics under steady-state conditions. A further test for both hypotheses can be conducted if the kinetic parameters obtained for both biotic pools of Figure 4-2 are compared to published information from soil and pure culture studies. Data obtained by Nelson *et al.* (1979) on the decomposition of cytoplasmic and cell wall materials showed that cell wall-C was oxidized faster than the cytoplasmic-C of some microorganisms but the reverse was true for other soil microbes. The data collected by the latter authors were kinetically analyzed by Juma and McGill (1986). They defined two components, a labile (k = week^{-1}) and a more resistant component ($k = 10^{-3}$ week^{-1}). Both compartments were observed during decomposition of either cytoplasmic or cell wall material, reflecting the role of biomass generation and the contribution of its subsequent turnover to ^{14}C dynamics during longer term incubations than used here. This evidence suggests that the biomass through which substrates flow, and physiological characteristics of it, rather than qualitative differences in substrates determine ^{14}C dynamics.

Bacteria and fungi can concentrate amino acids internally in a pool against concentration gradients (Mandelstam 1958; Bengtson, 1982). Studies in aquatic systems and pure cultures indicate that most amino acids are incorporated mainly into bacterial proteins (Ferguson and Sundra, 1984; Mandelstam, 1958). The accepted hypothesis (Figure 4-2B) comprises a pool of intermediates from which respiration occurs rapidly and a metabolite (possibly proteins) pool with slow turnover. This model describes the experimental observations of both ^{14}C in solution and evolved as CO_2 and is consistent with pure culture observations. It was not possible to distinguish two microbial populations under steady-state conditions on a kinetic basis. Similarly, the glucose data showed that controls on ^{14}C kinetics under non-perturbed conditions were more physiologic than taxonomic. This means either: 1) only one group of microorganisms contributes 2)both operate at similar rates under such nutrient limited conditions. Further, if nutrient supply is the major limitation, then relative growth rates are not a control. From the above it is concluded that two different taxa cannot be distinguished kinetically. By elimination, the two microbial moieties defined kinetically must reflect divergent metabolic functions for incorporated ^{14}C.

The following points favor a physiologic control on ^{14}C dynamics as described by the kinetic analysis:

1. Values for the turnover time of amino acid and protein pools of Figure 4-2B are consistent with literature values for those components in both fungal and bacterial cells (Table 4-5).
2. Protein turnover rate in exponentially growing cells is negligible, and about 5% per hour

in stationary growing cells (Rotman and Spiegelman, 1954; Koch and Larry, 1955; Mandelstam, 1963).

3. Microbial proteins include fast and slow cycling components (Alberghina and Martegani, 1977; Bachmair, 1986).
4. Degradation rates of total cell protein in fungi and bacteria are comparable (Mandelstam, 1963; Alberghina and Martegani, 1977).
5. The turnover rate (0.84 min^{-1}) for the amino acid pool of *E. coli* (Britten and McLure, 1962) is comparable to that of the microbial-1 pool in the Gray Luvisolic soil (0.67 min^{-1}).
6. Degradation of proteins in *E. coli* under stationary growth is about 5% $hour^{-1}$ (Mandelstam, 1960) and the rate of ^{14}C degradation for microbial-2 pool in the Luvisolic soil is 6% $hour^{-1}$.
7. Ratios of total protein/cytoplasmic amino acid in microorganisms range between 6 to 600 (Mandelstam and McQuillen,1973; Holden, 1962). The same ratio for the Luvisolic soil calculated from model output at steady-state (Figure 4-5) is 480.

The dynamics of ^{14}C-cystine through four pools in the Gray Luvisolic soil are shown in Figure 4-5. Initially, the labelled amino acid enters the cytoplasmic amino acid pool rapidly. Later when the specific activity of pool amino acid equals that present in the soil solution through exchange reactions, ^{14}C-cystine enters the cell at the same rate as it is utilized for protein synthesis. Similar processes have been observed for pool amino acids in *E. coli* (Britten and McLure, 1962). In addition, cystine is a more complex N and S source than ammonium or sulfate, hence the amino acid molecules are absorbed rapidly because they require less energy for incorporation into proteins, i.e. increases the Y_{ATP} values (g cells/mol ATP generated) in comparison to ammonium and sulfate as N and S sources (Ratcliffe *et al.* 1983). A further analytical test for this hypothesis may involve the use of ^{13}C NMR to study the incorporation of cystine-C into cell proteins or other macromolecules.

In conclusion, two kinetic models described the experimental data for ^{14}C cycling through soil solutions for perturbed and non-perturbed conditions. Both models are consistent with pure culture studies. The first model is consistent with the hypothesis that under perturbed conditions, glucose-C is allocated into a pool of small intermediate molecules of biosynthesis and into macromolecules such as proteins and RNA. The protein component does not degrade measurably over the period of study. The second kinetic model validated for non-perturbed soil conditions distinguishes ^{14}C-cystine allocation between microbial cytoplasmic and protein components. Neither model can be considered to distinguish between microbial taxa. Both sets of experimental conditions (i.e. excess glucose addition to favor growth, and minute cystine additions to preserve steady-state lead to the conclusion that physiologic rather than taxonomic controls determine short term ^{14}C kinetics in soils under laboratory conditions.

4.5 Bibliography

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Table 4-1. Means of ^{14}C evolved as CO_2 during six hours from two soils treated with ^{14}C-cystine plus streptomycin and actidione.

	^{14}C evolved as CO_2 (% of initial dose)[1]			
Soil	Cystine	(+Streptomycin)	(+Actidione)	(Streptocymcin +Actidione)
Gray Luvisolic	23 b	28 a	26 ab	29 a
D. Brown	21 b	22 ab	23 ab	26 a

[1] q_{sp} = 23,630 dpm

Within rows, numbers followed by different letters are significantly different from each other as judged by Duncan's multiple range test (p=0.05).

Table 4-2. ^{14}C evolved as CO_2 and remaining in the solution of a Gray Luvisolic soil supplemented with labelled cystine. Cystine-^{14}C flows through bacteria and fungi according to hypothesis of Figure 4-2a.

Time (min)	^{14}C (as % of initial dose)			
	Evolved as CO_2		Remaining in solution	
	Observed	Predicted	Observed	Predicted
0	0	0	100.0000	99.9800
5	1.4	1.8	0.3315	0.6735
15	4.4	6.2	0.1561	0.1420
30	10.6	11.4	0.1144	0.0139
60	14.6	17.1	0.0919	0.0007
90	16.1	19.7	0.0794	0.0004
120	19.1	21.3	0.0747	0.0005
180	21.4	23.5	0.0708	0.0004
240	25.9	25.3	0.0674	0.0007
360	29.6	28.8	0.0648	0.0001
X^2		2.1		45.1

$X^2 = 3.84$ at $P=0.05$.

Table 4-3. ^{14}C evolved as CO_2 and remaining in the solution of a Gray Luvisolic soil supplemented with labelled cystine. Cystine-^{14}C flows through microbial cytoplasm and proteins according to hypothesis of Figure 4-2B.

Time (min)	^{14}C (% of initial dose) Evolved as CO_2 Observed	 Predicted	 Remaining in solution Observed	 Predicted
0	0.0	0.0	100.0000	100.0000
5	1.4	3.4	0.3315	0.3325
15	4.4	6.0	0.1561	0.1648
30	10.6	9.0	0.1144	0.1089
60	14.6	12.5	0.0919	0.0903
90	16.1	15.6	0.0794	0.0857
120	19.1	18.6	0.0747	0.0820
180	21.4	20.6	0.0708	0.0751
240	25.9	24.9	0.0674	0.0688
360	29.6	31.3	0.0648	0.0570
x^2		1.13		0.0015

$x^2 = 3.84$ at $P=0.05$

Table 4-4. Estimated rate constant and model validation criteria to describe the transfer of glucose-C under growing conditions in a Gray Luvisolic soil.

Model Configuration	Rate Constant (h^{-1})								Model selection	
	k_1[1]	k_2	k_3	k_4	k_5	k_6	k_7	k_8	Variable[2]	Validaton[3]
Figure 4-1A	0.62	29.00	0.03	$5x10^{-4}$	N.A.[4]	N.A.	N.A.	N.A.	CO_2	rejected
Figure 4-1B	0.70	8.90	0.05	N.A.	N.A.	N.A.	N.A.	N.A.	CO_2	accepted
Figure 4-1C	0.65	0.04	4.90	10.40	N.A.	0.11	$2x10^{-4}$	N.A.	CO_2	accepted
Figure 4-1D	0.02	3.00	$1x10^{-3}$	0.04	$6x10^{-4}$	$2x10^{-1}$	$1x10^{-3}$	$2x10^{-4}$	CO_2	rejected
Figure 4-2A	5.01	$1x10^{-4}$	0.76	25.10	0.04	$6x10^{-6}$	N.A.	N.A.	CO_2	rejected
Figure 4-2B	$8x10^{-4}$	0.11	0.31	9.00	0.06	$3x10^{-7}$	N.A.	N.A.	CO_2	rejected

[1] Each k value represents the reaction defined by the respective model configuration.

[2] Variable used to compare model output with experimental values.

[3] The Chi-square (x^2) for goodness of fit test was used to validate the models.

[4] N.A. = reaction not applicable for the model.

Table 4-5. The turnover rates for amino acid and protein pools in microbial cells and soil components.

Microorganism	Type of Growth	Growing Substrate	Pool	Turnover rate (h^{-1})	Source
Escherichia coli	Exponential	^{14}C-valine	Amino acid	50.80	Britten and McLure (1962)
Escherichia coli	Exponential	^{14}C-glycine	Protein	0.00003	Koch and Larry (1955)
Escherichia coli	Exponential	^{14}C-Lactate	Protein	0.00005	Rotman and Spiegelman (1954)
Escherichia coli	Stationary	Complete	Protein	0.006	Willets (1967)
	Stationary	-Phosphate	Protein	0.048	
	Stationary	-glucose	Protein	0.053	
	Stationary	-(NH_4^+, leucine)	Protein	0.048	
	Stationary	+20 amino acids	Protein	0.016	
	Stationary	-Mg^{+2}	Protein	0.029	
Escherichia coli	Stationary	^{14}C leucine	Protein	0.050	Mandelstam (1963)
Neurospora crassa		Glucose or acetate	Protein	0.012	Alberghina ***et. al.*** (1977
		Ethanol	Protein	0.029	
			Fast protein	0.920	
			Slow protein	0.012	
Saccharomyces cerevisiae	Exponential	Galactose and Glucose	Fast protein	0.208	Bachmair ***et al.***, (1986)
			Slow protein	0.035	
Soil (Gray Luvisolic)	Stationary	^{14}C-Cystine	Microbial-1	40.20	Chapter 3
			Microbial-2	0.06	

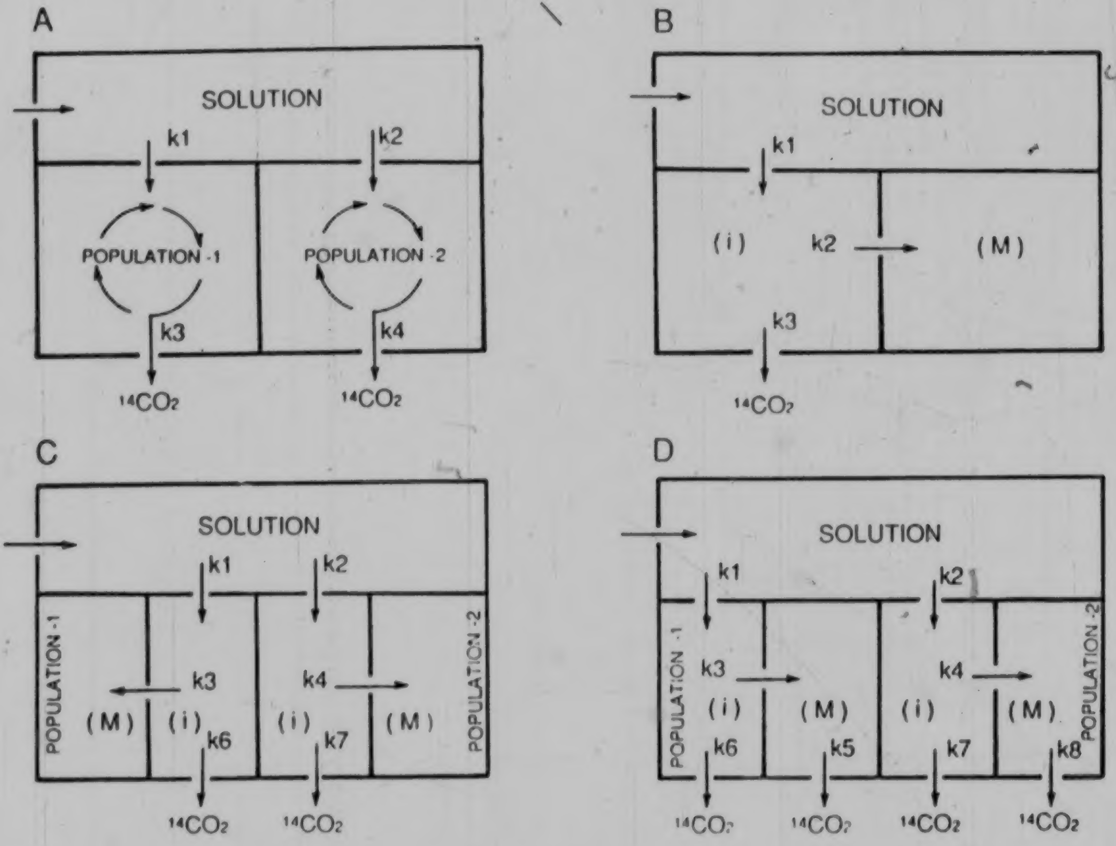
A
SOLUTION
k1
k2
POPULATION -1
POPULATION -2
k3
k4
$^{14}CO_2$
$^{14}CO_2$
B
SOLUTION
k1
(i)
k2
(M)
k3
$^{14}CO_2$
C
SOLUTION
k1
k2
POPULATION -1
k3
k4
(M)
(i)
(i)
(M)
POPULATION -2
k6
k7
$^{14}CO_2$
$^{14}CO_2$
D
SOLUTION
k1
k2
POPULATION -1
k3
(i)
(M)
k4
(i)
(M)
POPULATION -2
k6
k5
k7
k8
$^{14}CO_2$
$^{14}CO_2$
$^{14}CO_2$
$^{14}CO_2$

Figure 4-1. The dynamics of ^{14}C-glucose cycling through growing soil microorganisms after an excess of ^{14}C-substrate was added to a Gray Luvisolic soil.

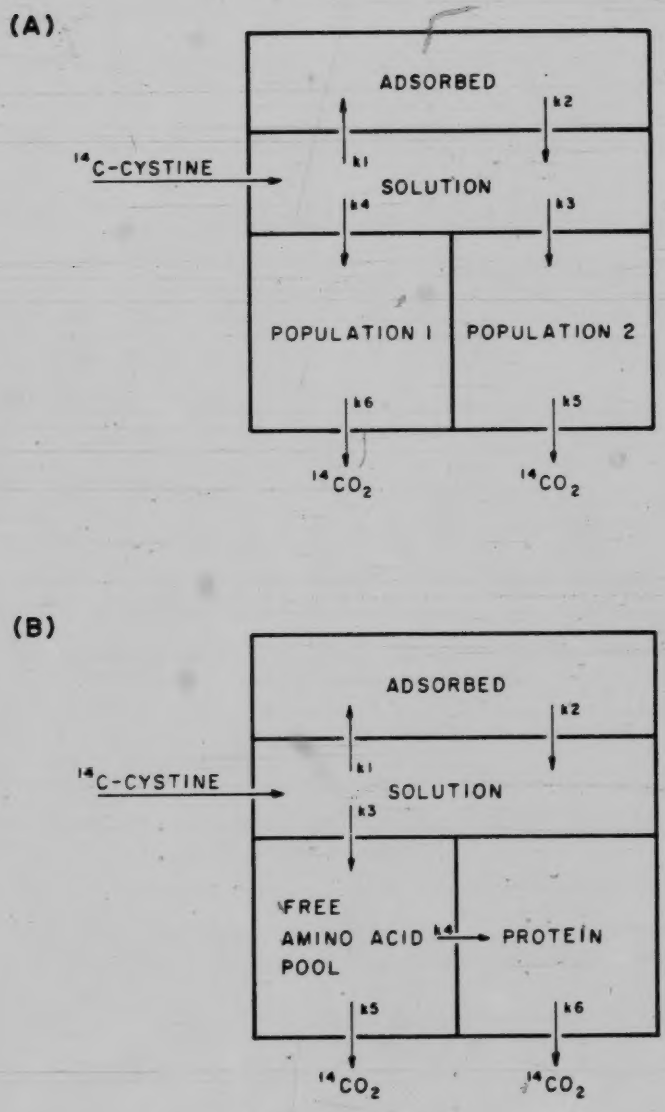
(A)
ADSORBED
k1
k2
^{14}C-CYSTINE
SOLUTION
k4
k3
POPULATION 1
POPULATION 2
k6
k5
$^{14}CO_2$
$^{14}CO_2$
(B)
ADSORBED
k1
k2
^{14}C-CYSTINE
SOLUTION
k3
FREE
AMINO ACID
POOL
k4
PROTEIN
k5
k6
$^{14}CO_2$
$^{14}CO_2$

Figure 4-2. Hypothesis for (A) cystine cycling through fungi and bacteria (b) cystine flowing into active cells to form part of the free amino acid pool and subsequent incorporation into proteins.

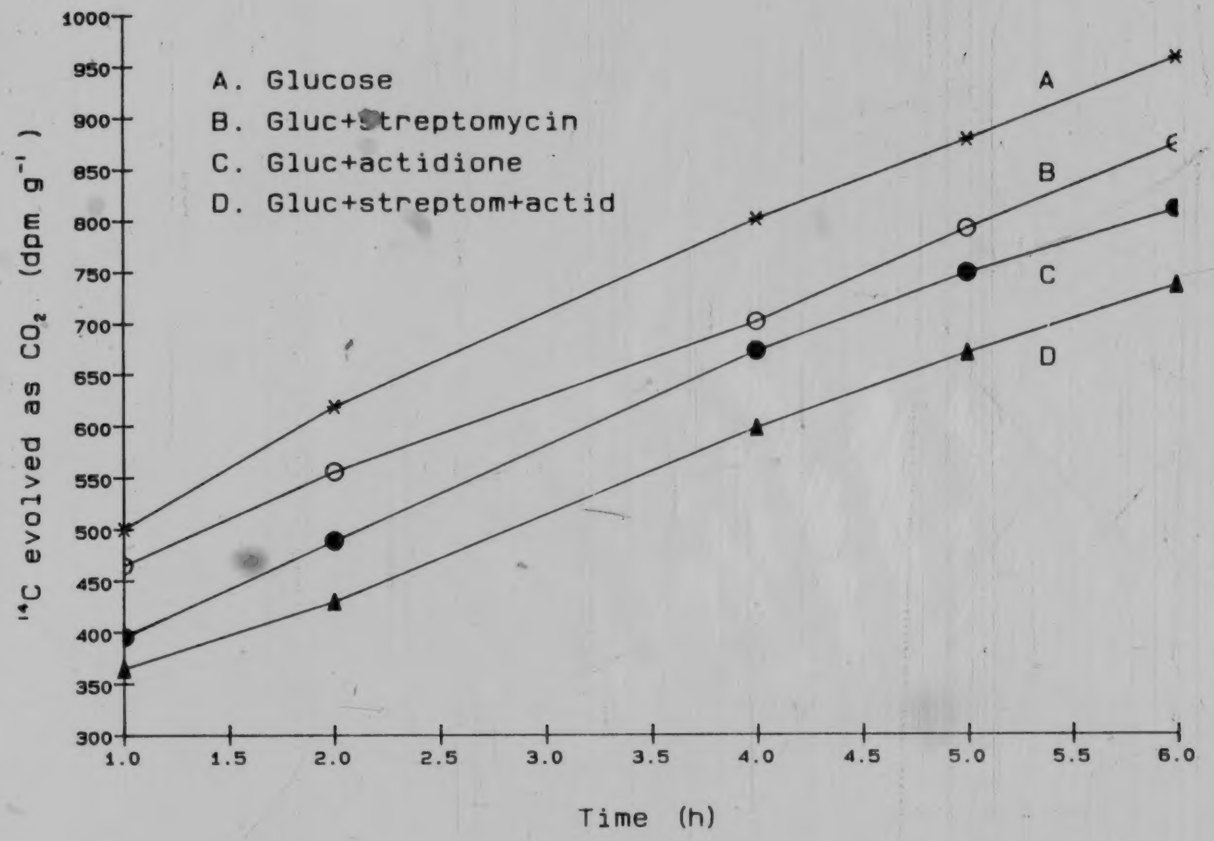

Figure 4-3 The effect of actidione (2000 $\mu g\ g^{-1}$) and streptomycin (1000 $\mu g\ g^{-1}$) on the respiration of ^{14}C-glucose (q_{so}) = 17,320 dpm) from a Gray Luvisolic soil.

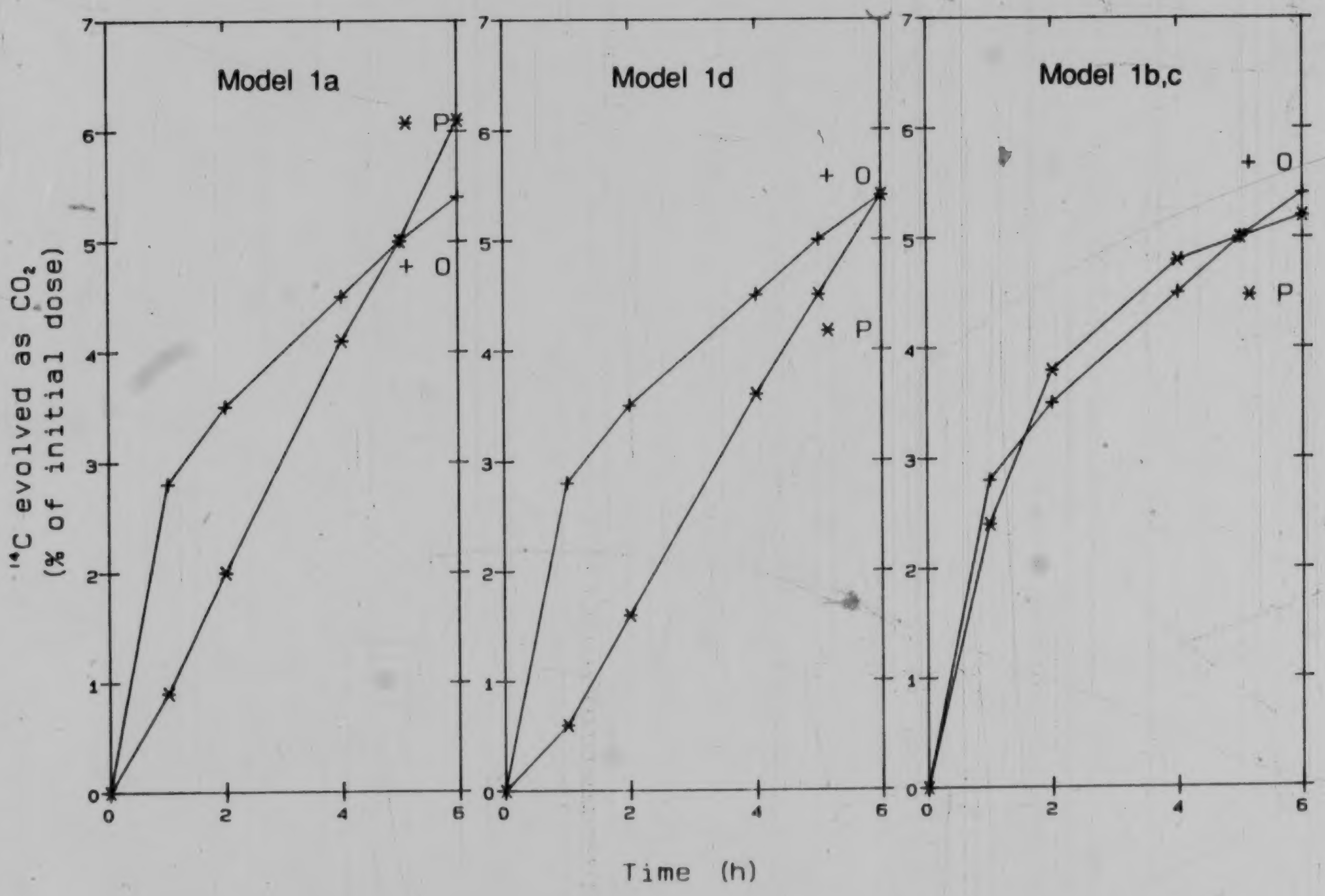

Figure 4-4. Simulation of $^{14}CO_2$ evolved from a glucose amended soil defined as 3 and 5 compartment models of Figure 4-1.

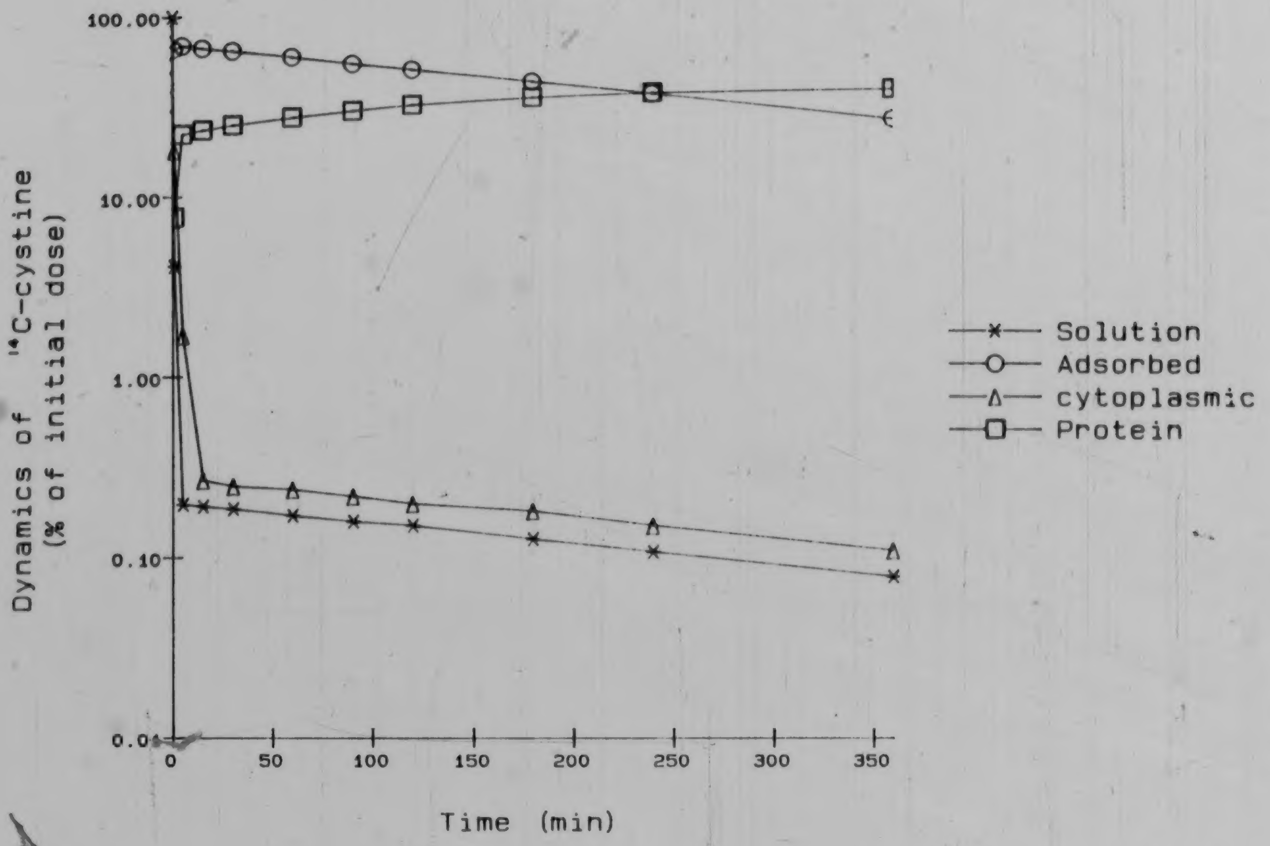

Figure 4-5. Kinetics of ^{14}C-cystine cycling in a Gray Luvisolic soil defined as the four pool system of Figure 4-2b.

5. THE DYNAMICS OF FREE CYSTINE CYCLING AT STEADY-STATE THROUGH THE SOLUTIONS OF SELECTED CULTIVATED AND UNCULTIVATED CHERNOZEMIC AND GRAY LUVISOLIC SOILS[1]

5.1 Introduction

Steady-states achieved by soils after thousands of years of soil formation are disrupted by cultivation. Such disruption results in losses of soil organic matter (Campbell and Souster, 1982), less microbial biomass-C (Voroney *et al.* 1981) and lower fertility (P.F.R.A., 1983). Understanding of such changes has benefited from long term experiments (Jenkinson, 1965) or perturbation studies (Paul, 1984) together with simulation modelling of processes on a daily time step (McGill *et al.* 1981; Paul and Juma, 1981; Van Veen *et al.* 1985), which have shown the important function of soil organisms in organic matter dynamics.

Kinetic analyses of microbial biomass processes *in situ* provide information which is not available from fractionation studies (Juma and McGill, 1986). Rather than using long term incubations, short term kinetic studies describe the flows of cystine-C under steady-state conditions. Such kinetic analyses have not been extensively applied in soil organic matter investigations. Chapter 3 developed a model for short-term dynamics of cystine using kinetic analyses. The model involves two biontic pools which were associated with microbial cytoplasmic amino acids and protein pools (Chapter 4). Interactions among soil components may influence processes and reactions of internal cycling of soluble substrates through soil solutions under steady-state conditions.

Comparative analyses of soils having different histories of either management or soil formation provide information on controls affecting the dynamics of organic substrates through soil solutions. Within this context, cystine cycling is compared between pairs of cultivated and uncultivated soils from different environments with the objectives of determining the effects of past soil cultivation on internal cycling of soluble cystine.

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[1] A version of this chapter will be submitted for publication. C.M. Monreal and W.B. McGill (Soil Biology and Biochemistry).

5.2 Materials and methods

5.2.1 Soils

Eight soils corresponding to a climosequence were used. The soils were cultivated and uncultivated Black, Dark Brown and Brown Chernozemic soils and a Luvisolic soil cropped to two rotations: i) wheat-fallow rotation (2y); and ii) wheat, oats, barley, forage, forage (5y). All soils were from central and southern Alberta. The soil physical and chemical characteristics are described in Chapter 2 and McGill *et al.* (1986).

5.2.2 Incubation of soil samples

Duplicated one gram (oven dry basis) soil samples were incubated with labelled cystine added at a rate of 60 ng g^{-1} soil. The protocol of experiments was described in Chapter 3.

5.2.3 Experimental design

Incubation studies were designed as factorial experiments, where soils, cultivation and time were the main factors. Analysis of variance (ANOVA) was conducted on the ^{14}C remaining in solution and evolved as CO_2 over time. Duncan's multiple range and the least significant difference tests were applied to determine statistical differences (Zar, 1984).

5.2.4 ^{14}C-Carbonates

The amount of ^{14}C-carbonates originating from microbial respiration was determined as follows: immediately after collecting the $^{14}CO_2$, one soil cup replicate was transferred into a second scintillation vial containing 0.2 M NaOH. The vial was sealed with a rubber septum and 1 ml of 4 M HCl was injected through the septum into the cup containing the soil sample. Evolved $^{14}CO_2$ was collected for 5 minutes and the ^{14}C activity determined. Vials left to react for 10 minutes showed no further $^{14}CO_2$ evolution. Three different soils were chosen to represent conditions of different pH in the soil solution.

5.2.5 Microbial biomass-^{14}C

Microbial biomass-^{14}C was estimated in samples used for $^{14}CO_2$ by the chloroform fumigation method of Jenkinson and Powlson (1976).

5.2.6 The model

Kinetic analysis has defined four soil components and reactions affecting the cycling of cystine in a Gray Luvisolic and an Andept soil (Chapter 3). The basic model accepted for the Luvisolic soil comprises the soil solution, adsorbed phase and two microbial pools (Figure 5-1B). The two biontic pools were associated with cytoplasmic free amino acids and cell proteins (Chapter 4). Alternate hypotheses of Figures 5-1a and 5-1c were also retested to confirm the generality of 5-1b in describing cycling of cystine-C through the solutions of eight soils.

5.2.7 Model selection and numerical analysis

The steps of numerical analysis for model selection used BMDPAR and CSMP. These steps were described earlier in Chapter 3. Measured values for the ^{14}C remaining in solution and evolved as CO_2 were compared to those simulated by models of Figure 5-1. A model was accepted if experimental and observed values were not different as determined by a Chi-square test for goodness of fit.

5.2.8 Rate constant and pool size

These parameters were calculated as described in Chapter 3. Pool size for solution cystine in cultivated and virgin Dark Brown Chernozemic and the 2 year rotation Luvisolic soil were given by Chapter 2.

5.3 Results

5.3.1 ^{14}C remaining in solution

The activity of ^{14}C remaining in the solution of all soils after 6 hours was $< 1\%$ of the initially added dose. The analysis of variance showed no significant differences between soils in ^{14}C activity remaining in solution (Table 5-1).

5.3.2 ^{14}C evolved as CO_2

Labelled CO_2 production approached a plateau in every soil within 6 hours of incubation. The plateau ranged between 14.1 and 29.6% in the virgin Black Chernozemic and the Luvisolic soil (2 year rotation), respectively.

Cultivated soils evolved significantly more CO_2 than their respective virgin samples (Figure 5-2a,b,c). Within the Chernozemic order, the amount of ^{14}C evolved as CO_2 from the cultivated samples tended to increase in the following order: Brown < D. Brown < Black. Conversely, the virgin samples corresponding to the Brown soils evolved significantly more CO_2 than did the Dark Brown or Black virgin samples. The amount of $^{14}CO_2$ respired from the Luvisolic 2 year rotation samples was similar to that evolved from the 5 year rotation samples (Figure 5-2D).

5.3.3 Dissolved $^{14}CO_2$ in the soil solution

Samples of four soils encompassing the range of pH found in all eight were examined to determine the extent to which the ^{14}C oxidized to CO_2 was transformed into carbonates, which would cause CO_2 evolution to underestimate total CO_2 production.

Treatment with 4 M HCl following CO_2 collection during incubation released less than 0.2% of the initially added ^{14}C (Table 5-2). This release was complete within five minutes. Beyond this time, negligible or no ^{14}C was detected as CO_2. It was concluded that $^{14}CO_2$ measurements did not underestimate total CO_2 production by soil microorganisms (Table 5-2).

5.3.4 Microbial biomass-^{14}C

Several soil samples were fumigated with chloroform to confirm ^{14}C had been incorporated into soil organisms. Biomass-^{14}C for different soils ranged between 54 and 75% of the initially added ^{14}C (Table 5-3). The ^{14}C in microbial cells varied but there were no obvious trends for the period starting at 60 minutes and ending after 6 hours of incubation.

5.3.5 Model selection for the cycling of cystine in cultivated and virgin soils

The four compartment model of Figure 5-1B was accepted after its output mimicked the ^{14}C remaining in solution and evolved as CO_2 over time in all soils. The output for model 5-1b is consistent with the hypothesis that ^{14}C is allocated into cytoplasmic materials and cell proteins. One example of the model validation is represented by results obtained with the cultivated Black Chernozemic samples (Figure 5-3). Test of models 5-1a and 5-1c, respectively overestimated and underestimated experimental CO_2 values.

5.3.6 Rate constant and turnover time

The average overall turnover rate constant ± standard deviations for five pools decreased in the following order: soil solution, 3.0 ± 0.6 min^{-1} > cytoplasmic free amino acid pool, 0.64 ± 0.09 min^{-1} > adsorbed, 0.016 ± 0.005 min^{-1} > cell proteins, 0.0007 ± 0.0002 min^{-1} (Table 5-4). The turnover rate for the cytoplasmic pool is always faster in cultivated than in virgin Chernozemic soil samples. Although no other pool showed this consistent trend between virgin and cultivated samples, the adsorbed pool tended to be less dynamic in cultivated Chernozemic soils and in the more frequently cultivated (2y) Luvisolic soil. In cultivated samples the turnover rate for the solution and cell proteins is fastest in the Brown > Dark Brown > Black (Table 5-4).

The kinetic model proposes that microbial uptake (k_3) and adsorption (k_1) rates are the prevalent reactions transfering ^{14}C from solution. The adsorption rate (k_1) exceeded uptake rate (k_3) by an average of 3 fold. The oxidation rate from the cytoplasmic pool (k_5) exceeded the protein oxidation rate (k_6) by an average of 244 fold. In the Luvisolic soil every reaction rate, with the exception of the rate of desorption (k_2), is equal to or greater in the 2 year rotation than in the 5 year rotation samples. Also, the solution and protein pools cycle faster in the Luvisolic 2 year than in the 5 year rotation samples (Table 5-4).

In summary, the kinetic model of Figure 5-1B describes the experimental observations. Cultivated samples evolved more $^{14}CO_2$ than their respective virgin samples. Within the Chernozemic order the $^{14}CO_2$ evolved from virgin samples was greater in the Brown > D. Brown > Black. Differences in $^{14}CO_2$ evolution from virgin soil samples are coupled to the solution turnover rate. This rate was faster in the Brown > D. Brown > Black samples. The rate of C oxidation from the cytoplasmic pool in cultivated samples is greater than the oxidation rate from protein-C.

5.4 Discussion

Most soil organic matter transformation studies describe the changes of soil variables such as CO_2 respiration over time. Processes producing differences in the magnitude of such variables are, however, frequently undefined. Within this context, the present study attempts to describe mechanisms yielding differences in CO_2 respiration from soils having different management and pedogenic histories. The present four component model explains the experimental data and includes two microbial components. Chapter 4 associated the two biontic pools with cytoplasmic amino acids and intracellular proteins. The kinetic model linked the two microbial components to metabolic components rather than to different microbial taxa.

Significant differences in $^{14}CO_2$ evolution were observed between cultivated and virgin samples and between samples corresponding to different soil groups. Dormaar (1975) conducted an organic matter decomposition study in native Prairie soils of Canada. The amounts of CO_2 respired per g soil C was greater in Brown > D. Brown > Black samples. When ^{14}C-glucose was added to the former soils, a similar trend in $^{14}CO_2$ was observed among the soil groups. Dormaar (1975) attributed such differences in CO_2 respiration between soil groups to effects caused by different plant species inhabiting those soils. In the present experiments, the oxidation of ^{14}C-cystine to CO_2 from virgin samples exhibited the same trend among the Brown, Dark Brown and Black samples. The present kinetic model is consistent with these experimental observations. Further, the model associates the higher $^{14}CO_2$ respiration from Brown samples with a faster turnover of solution cystine.

Decomposition of labelled organic matter to CO_2 is slower in planted than in fallow soils and these C losses were related to microbial processes (Reid and Goss, 1983). In the present experiments, more $^{14}CO_2$ was respired from cultivated than from virgin samples. In all soils, solution cystine-C is adsorbed to colloids and is rapidly transfered into microbial cells where it is incorporated into proteins (Figure 5-4). After 6 hours, microbial proteins become the major sink for cystine-C based on calculations of steady-state pool sizes (Table 5-5). The biomass-^{14}C values obtained through $CHCl_3$ fumigation and those simulated after 6 hours of incubation are in agreement. This consistency shows up in spite of the fact that the model prediction is based on short six hour experiments and the estimate of biomass-C by $CHCl_3$ fumigation takes twenty days of incubation. About 80% of the $^{14}CO_2$ evolved from the cultivated samples originated from the rapidly cycling free amino acid pool (Figure 5-5). In contrast, 70% of the ^{14}C-CO_2 evolved from the virgin samples originated from the slower cycling protein pool (data not shown). The relation: microbial-^{14}C/(microbial-$^{14}C+CO_2$) estimates efficiency of substrate utilization by soil microbial biomass. Microbial-^{14}C was estimated from simulation modelling. These values expressed as % of initial dose were 38, 32 and 45 for the cultivated Brown, Dark Brown and Black soils, respectively. The simulated microbial-^{14}C values in virgin Brown, D Brown and Black soils were 56, 75, and 46%, respectively. On average, the efficiency of cystine utilization in cultivated soils and virgin samples was 60% and 76%, respectively. The CO_2 metabolic sources and the substrate utilization efficiencies are related to differences in oxidation rates between cultivated and virgin soils.

McGill *et al.* (1981) reported losses of up to 50% of the organic-C from cultivated soils in the last 100 years across the Canadian Prairies. For the soils used in this study a potential relation may exist between C incorporation into the protein pool and losses from the microbial cytoplasmic pool on one hand and cultivation on the other. If this is true, short term incubation studies and associated modelling may provide reliable information about the effects of soil management on the long term soil organic matter content. As such this approach could be a valuable tool for soil conservation/management studies.

In conclusion, the present kinetic model described cystine cycling in eight different soils. The model results were interpreted on the basis of the role of soil components and C allocation mechanisms influencing CO_2 respiration from soils with different management and pedogenic histories. The greater $^{14}CO_2$ evolution from the virgin Brown Chernozemic soil was associated with a faster turnover of solution cystine. The differences in $^{14}CO_2$ evolution between cultivated and virgin samples are attributed to different C allocation patterns among microbially mediated processes. In cultivated samples, the main sink for ^{14}C-cystine is the protein pool and the main source for $^{14}CO_2$ evolution is the cyoplasmic component. In contrast, a greater proportion of ^{14}C is oxidized from the more stable protein pool in virgin samples. Adsorption did not explain the differences between soils in $^{14}CO_2$ evolution and ^{14}C remaining in solution.

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Table 5-1. Mean values (% of initial dose) for ^{14}C remaining in solution and evolved as CO_2 from eight soils incubated during 6 hours following addition of ^{14}C-cystine.

	Chernozemic						Gray Luvisolic	
	Brown		Dark Brown		Black			
Variable	C[1]	V	C	V	C	V	2y	5y
Solution	0.11 a[2]	0.05 a	0.11 a	0.04 a	0.18 a	0.80 a	0.06 a	0.11 a
CO_2	23.1 b	21.1 ab	24.3 b	19.8 c	27.9 a	14.4 d	29.6 a	27.3 a

[1] C = Cultivated, V = Virgin.

[2] Within each row values not followed by the same letter are significantly different (p=0.05) from each other as judged by Duncan's multiple range and the Least significant difference tests.

Table 5-2. Microbial respiration of ^{14}C-cystine and the release of $^{14}CO_2$ from carbonates.

Soil	Gray Luvisolic				Brown Chernozemic		Black Chernozemic	
	2 year rotation		5 Year rotation		Vauxhall - Cultivated		Malmo - Virgin	
Time (min)	CO_2	Carbonates	CO_2	Carbonates	CO_2	Carbonates	CO_2	Carbonates
5	846	0	1302	87	2823	259	1943	66
10	2711	0	3235	0	6927	0	3128	0
30	7898	0	7911	0	11627	0	4437	0
60	11063	3	9075	0	17042	40	8266	0
120	13195	0	11640	0	26357	0	14136	7
180	14721	0	13794	0	32500	20	18855	14
240	16190	0	16438	0	36090	0	22484	0
360	17933	2	18712	7	42000	5	28531	0
q_{so} (dpm)*	62814		66096		193262		198268	

q_{so} = Amount of radioactivity (disintegrations per minute) of initial dose.

[1] The soil pH measured in a 1:2 soil to 0.01 M $CaCl_2$ suspension was: Gray Luvisolic (2y) = 6.9; Brown Chernozemic, cultivated = 7.2; Gray Luvisolic (5Y) = 6.8; Black Chernozemic, virgin = 6.5.

Table 5-3. The recovery of ^{14}C-cystine (± standard deviation) as microbial biomass in different soils and at different time.

Time (min)	Biomass-^{14}C (% of initial dose)[1]				
	Brown		Dark Brown		Black
	V[2]	C	V	C	C
60	60 ± 6	74 ± 5	68 ± 12	60 ± 11	67 ± 6
90	67 ± 5	70 ± 6	66 ± 8	70 ± 4	60 ± 7
120	71 ±10	57 ± 4	65 ± 6	58 ± 8	54 ± 4
240	72 ± 9	57 ± 4	70 ± 12	58 ± 7	62 ± 9
360	72 ± 6	60 ± 5	66 ± 5	60 ± 13	57 ± 5

[1] Labelled cystine was added at time zero at a dose equivalent to 195,000 dpm.

[2] V = virgin, C = cultivated.

Table 5-4. Turnover rates and rate constants for describing internal cystine cycling for model 5-1b in four soils with two management treatments each.

		Brown		Dark Brown		Black		Gray Luvisolic	
Parameter		C[1]	V	C	V	C	V	2y	5y
Compartment		Turnover rate (min^{-1})							
Soil solution,		3.4	3.6	3.3	3.4	3.0	1.8	3.0	2.6
Cytoplasmic cystine,		0.67	0.59	0.76	0.49	0.76	0.54	0.65	0.66
Adsorbed,		0.013	0.013	0.013	0.023	0.017	0.024	0.009	0.019
Protein,		0.0007	0.0006	0.0006	0.0008	0.0002	0.0008	0.001	0.0005
Reaction		Rate Constants (min^{-1})							
Adsorption,	(k_1)	2.8	2.6	2.8	2.1	2.4	1.6	2.1	2.0
Desorption,	(k_2)	0.013	0.013	0.013	0.023	0.017	0.024	0.009	0.019
Microbial uptake,	(k_3)	0.58	0.98	0.49	1.28	0.58	0.18	0.90	0.55
Protein synthesis,	(k_4)	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Cystine respiration,	(k_5)	0.186	0.106	0.276	0.008	0.280	0.055	0.180	0.180
Protein respiration,	(k_6)	0.0007	0.0006	0.0006	0.0008	0.0002	0.0008	0.001	0.0005

[1] C = cultivated, V = virgin.

Table 5-5. Pool size and flow rate values for Cystine-C cycling at steady-state through three soils defined as the four compartment model of Figure 5-1B.

	Dark Brown		Gray Luvisolic
	C	V	(2y)
		Pool Size (Q, ng C g^{-1})	
Solution	54	102	111
Cytoplasmic	35	268	154
Adsorbed	11631	10200	25900
Protein	28000	160800	73920
		Flow Rates (F, ng C min^{-1} g^{-1})	
Adsorbed (F_1)	151	235	233
Desorbed (F_2)	151	235	233
Microbial uptake (F_3)	26	131	100
Protein synthesis (F_4)	17	129	74
Cystine oxidation (F_5)	10	82	26
Protein oxidation (F_6)	17	129	74

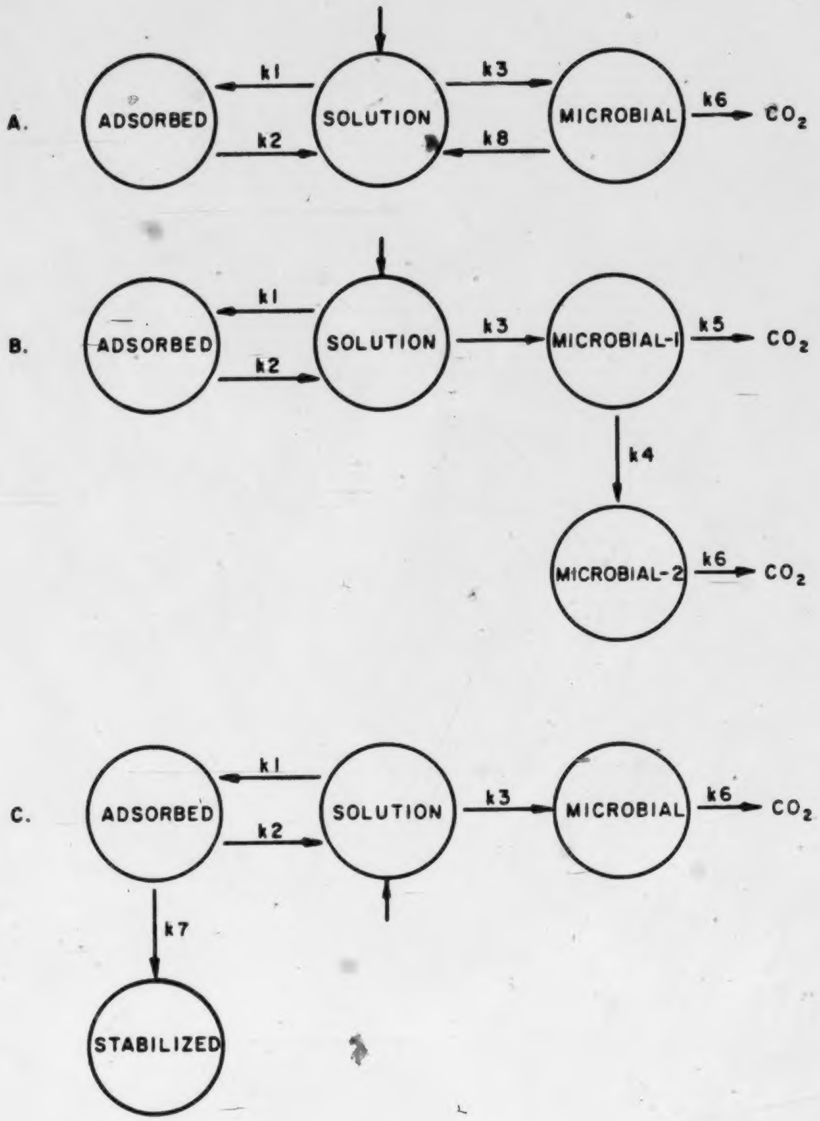
A.
ADSORBED
SOLUTION
MICROBIAL
k1
k2
k3
k8
k6
CO2
B.
ADSORBED
SOLUTION
MICROBIAL-1
MICROBIAL-2
k1
k2
k3
k4
k5
k6
CO2
CO2
C.
ADSORBED
SOLUTION
MICROBIAL
STABILIZED
k1
k2
k3
k6
k7
CO2

Figure 5-1. Hypotheses representing the cycling of free cystine through a three and four compartment soil ecosystem.

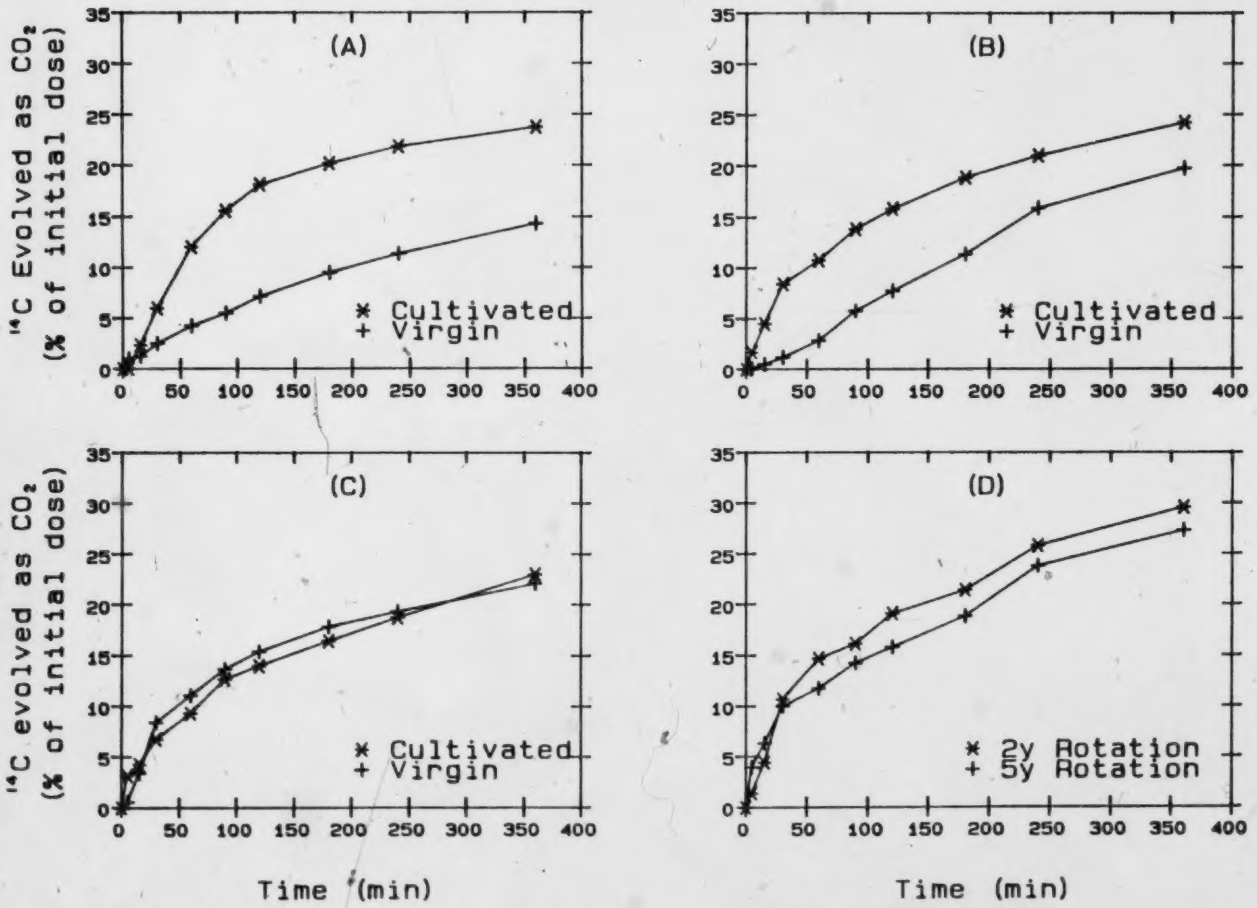

Figure 5-2. The respiration of ^{14}C-cystine in a Black (A), Dark Brown (B), Brown (C) Chernozemic soils and in Gray Luvisolic (D) soil samples.

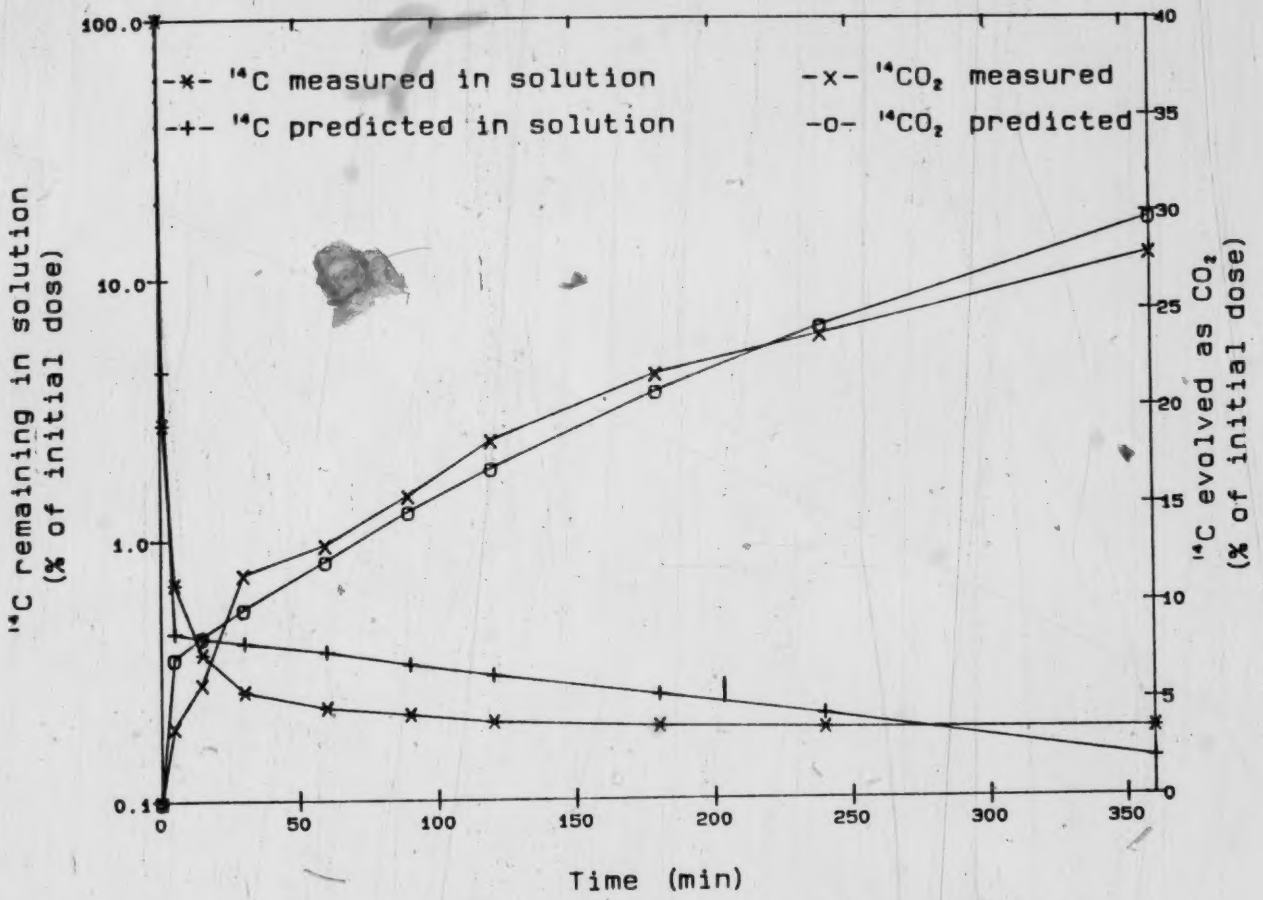

Figure 5-3. The measured and predicted ^{14}C remaining in solution and evolved as CO$_2$ in a Black Chernozemic soil defined as the four compartment model of Figure 5-1b.

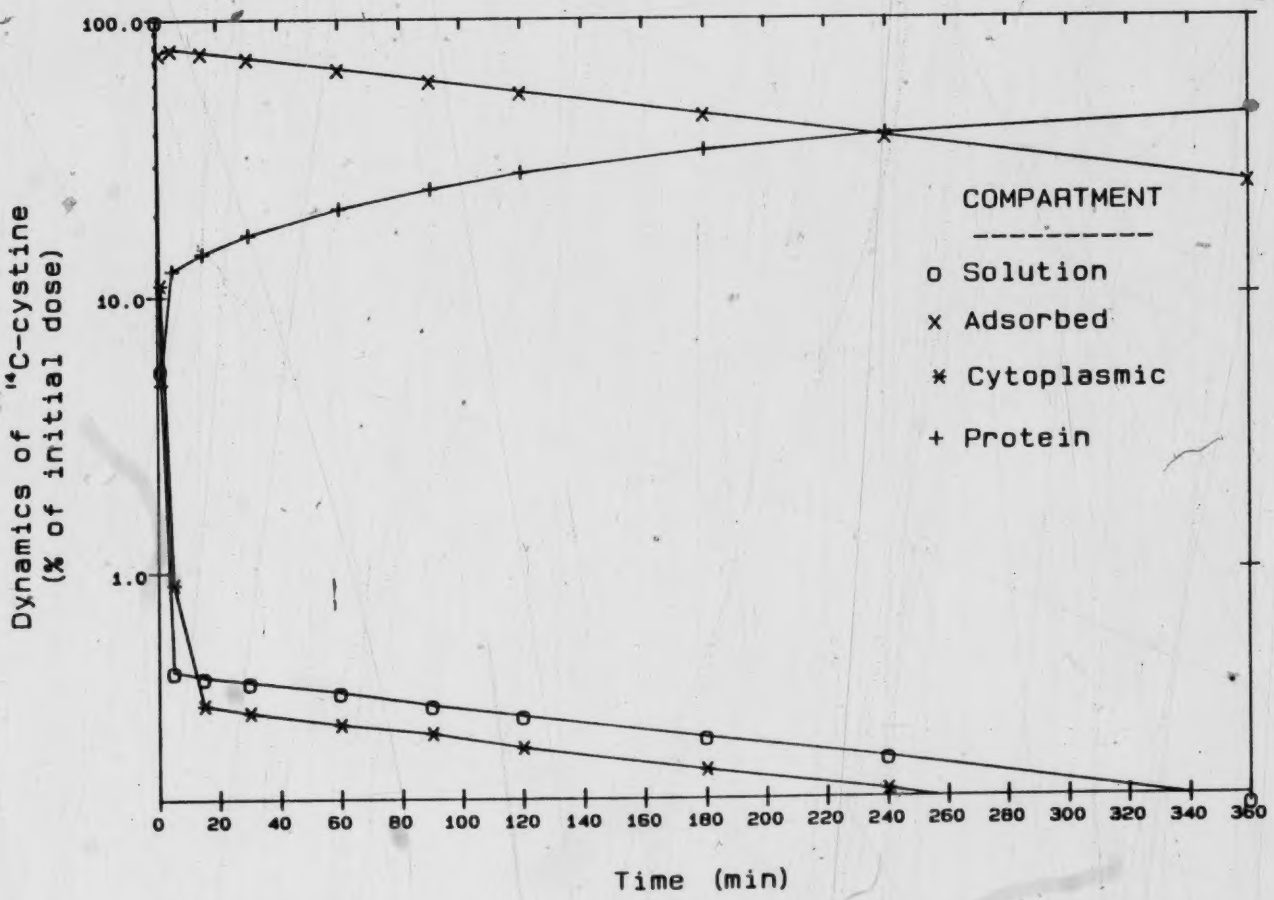

Figure 5-4. The dynamics of ^{14}C-cystine in a cultivated Black Chernozemic soil described by the 4 compartment model of Figure 5-1b.

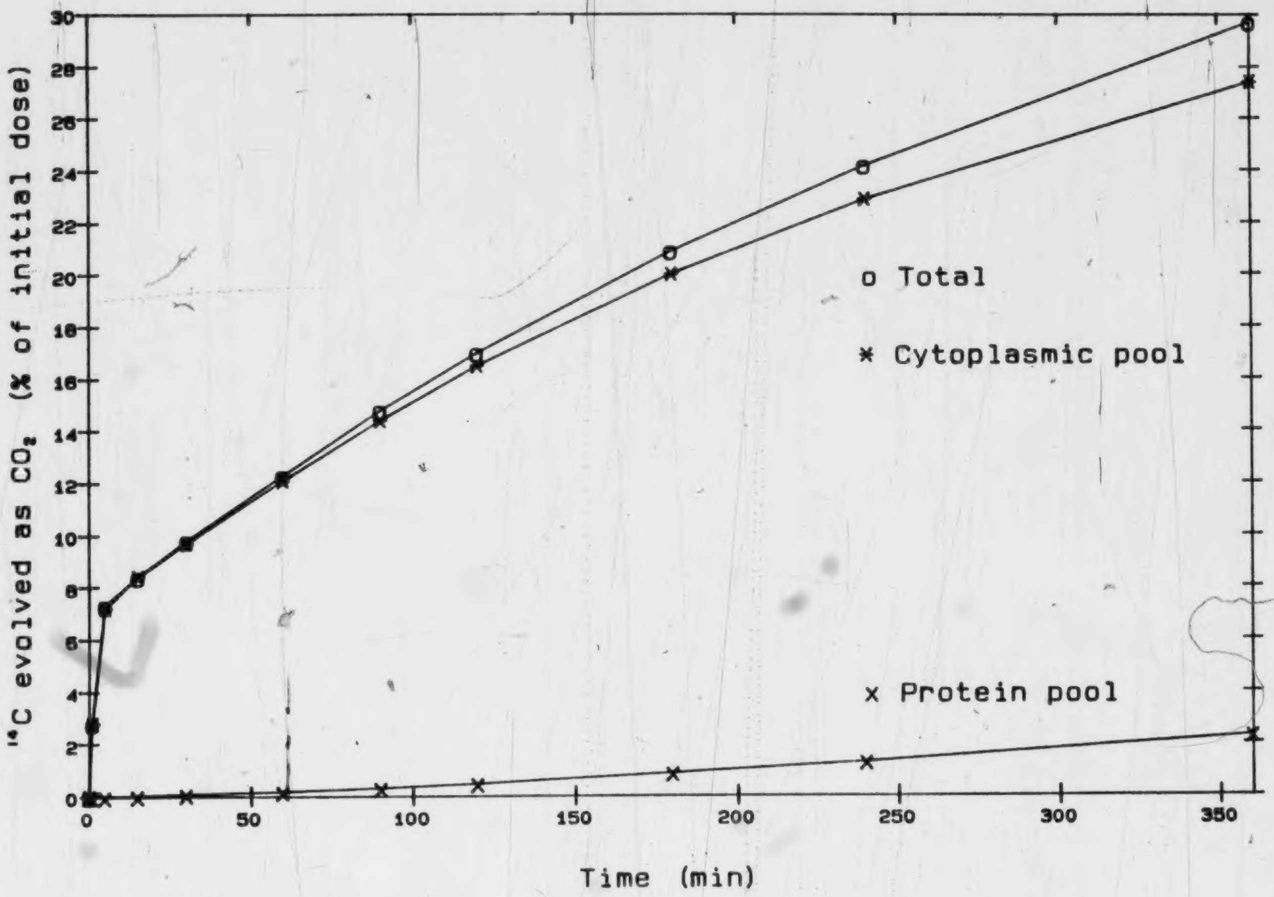

Figure 5-5. Sources of predicted ^{14}C evolved as CO_2 from two microbial components of a cultivated Black Chernozemic soil.

6. THE EFFECTS OF SOIL AMENDMENTS ON THE DYNAMICS OF FREE CYSTINE CYCLING AT STEADY-STATE THROUGH THE SOLUTIONS OF A BLACK CHERNOZEM AND AN ANDEPT SOIL[8]

6.1 Introduction

Decomposition of soil organic matter and plant residues such as cereal straw and alfalfa hay are affected by the addition of fertilizers (Harmsen and Kolenbrander, 1965; McGill *et al.* 1981), level of substrate (Stotsky and Norman, 1961), irrigation (De Jong *et al.* 1974), cultivation (Chapter 5), the environmental quality surrounding microbial cells (Zunino *et al.* 1982), and by microbial interactions (Woods *et al.* 1982). Explanations describing the effects caused by different soil amendments on CO_2 evolution are inconclusive and unsatisfactory (Söderstrom *et al.* 1983).

Kinetic models used in Chapters 3 and 4 separated and identified abiotic and microbial pools controlling the internal cycling of free cystine in allophanic and non-allophanic soils. Two microbial pools were associated with cytoplasmic aminoacids and intracellular proteins in non-allophanic soils at steady-state (Chapter 4). Both physiologic components controled cystine dynamics in eight such soils (Chapter 5). In one allophanic soil (Andept) dynamics were controled by one physiologic pool (analogue to proteins) and abiotic stabilization reactions. The kinetic model associated differences in ^{14}C evolved as CO_2 between non-allophanic soils with different pedogenic and management histories (Chapter 5).

With this background the present study uses kinetic analysis and modelling to determine the effects of inorganic and organic amendments on the internal cycling of free cystine in one allophanic and one non-allophanic soil.

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[8] A version of this chapter will be submitted for publication. C.M. Monreal and W.B. McGill (Soil Biology and Biochemistry).

6.2 Materials and methods

6.2.1 Soils

A cultivated Black Chernozemic (Malmo CL, Canada) and an Andept cultivated soil (Santa Barbara C, Chile) were used in this study. Other soil characteristics were described in Chapter 2.

6.2.2 Protocol of experiments

Incubation studies with ^{14}C-cystine and experimental measurements of ^{14}C remaining in solution and evolved as CO_2 were described in Chapter 3.

6.2.3 Amendments

Barley straw (0.4% N, 0.09% P) and alfalfa hay (4.3% N, 0.3% P) were added at the beginning of a 14 day preincubation period at a rate of 2% (w/w). Glucose, the amino acid mixture and inorganic fertilizer salts were mixed and added with the ^{14}C-cystine at the start of each incubation. For each soil, the volume of solution added, brought soil moisture to a potential of -33 kPa. Glucose (Fisher Scientific) and a standard mixture containing 5.6 μg $\mu l^{-1}$of each of 19 amino acids used for amino acid autoanalyzers (Sigma) were added at a rate of 30 and 5 μg of each amino acid g^{-1} soil, respectively. Nitrogen, sulphur and phosphorus were added as NH_4NO_3, K_2SO_4 and KH_2PO_4 (Fisher Scientific, reagent grade) at a rate of 84.6, 40 and 122 μg compound g^{-1} soil, respectively. Note that K was incidentally added.

6.2.4 Statistical design

Incubation studies were designed as factorial experiments, with soils, amendments and time defined as the main factors. Analysis of variance (ANOVA) was conducted on the ^{14}C remaining in solution and evolved as CO_2 over time. Duncan's multiple range and the least significant difference tests were used to determine statistical differences between treatments

(Zar, 1984).

6.2.5 The models

The models of Figure 6-1 were used to describe the cycling of cystine in soils of Canada and an Andept of Chile (Chapter 3). The two biontic pools of Figure 6-1B have been associated with microbial cytoplasmic amino acids and proteins (Chapter 4). Model 6-1C proposes that cystine is partially protected against microbial attack by stabilization reactions of the substrate with soil minerals.

6.2.6 Model selection and numerical analysis

Values for ^{14}C remaining in solution and evolved as CO_2 were compared to those simulated by models 6-1A, B and C. A model was accepted if the observed and pedicted values were not different as determined by a Chi-square test for goodness of fit. The steps of numerical analysis for model selection used BMDP3R, BMDPAR and CSMP (Chapter 3).

6.3 Results

Experiments were carried out to determine the effects of organic and inorganic amendments on the internal cycling of free cystine. Addition of plant residues and solutions containing N, P, and S separately or in combination were intended to simulate the effects of practices such as straw or green manure incorporation and fertilization. Addition of glucose and the mixture of amino acids simulate their release from cellulose and proteins, respectively.

6.3.1 ^{14}C remaining in solution

The ^{14}C activity remaining in solution varied between 0.02 and 0.24% in all treated soil samples after six hours. The ANOVA showed no significant differences in solution-^{14}C between the treatments (Table 6-1).

6.3.2 ^{14}C evolved as CO$_2$

The addition of amendments significantly affected the $^{14}CO_2$ evolution from both soils (Table 6-1). Every amendment increased the $^{14}CO_2$ evolved from the Black Chernozemic soil samples. The largest proportion of ^{14}C was respired in the straw and alfalfa amended Black samples, (36.5 to 39.4% of initial dose), respectively (Figure 6-2A, B, C).

Amendment of Andept samples with alfalfa, barley straw, NPS and glucose did not affect the $^{14}CO_2$ evolution. Addition of N, P, S, NPS and a solution containing 19 amino acids significantly inhibited the oxidation of ^{14}C-cystine to CO$_2$ from the Andept soil (Figure 6-3A, B, C).

6.3.3 Model selection

Model 6-1B described the Chernozemic samples. Its output closely simulated experimental CO$_2$ and solution values over time (Table 6-2). Output of model 6-1a and 6-1c overestimated and underestimated, respectively $^{14}CO_2$ respiration from the Chernozemic treated samples. Model 6-1b was also the only model that described the experimental data obtained from Andept samples pretreated with alfalfa hay and barley straw (Table 6-3). The observed ^{14}C remaining in solution and respired from Andept samples treated with N, P, S, NPS, the amino acid mixture and glucose were consistent only with the output of model 6-1C (Table 6-4). Model 6-1a overestimated the experimental $^{14}CO_2$ respiration from all amended samples.

6.3.4 Rate constant

Addition of amendments to the Chernozemic samples increased turnover rates for the solution and protein pools. Conversely, the turnover rate of ^{14}C in the cytoplasmic and adsorbed pools was reduced. Respiration rates from the cytoplasmic and protein pools were the most sensitive reactions to the addition of amendments (Table 6-5). The half-life for the protein pool in the Chernozemic soil changed from 58 hours in the control samples to 4 and

12 minutes in the alfalfa and straw amended samples, respectively.

Untreated and treated Andept samples with the amino acid mixture, N, P, S and NPS resulted in apparent adsorption reactions between ^{14}C-cystine and active organoallophanic groups. The effect of glucose and NPS on cystine cycling is similar to that shown by Andept control soil samples (Chapter 3). Abiotic stabilization generated a cystine-C pool with no detectable turnover during the six hour period. The kinetic model also indicates the latter amendments increased the turnover rate of solution, adsorbed and microbial proteins, but decreased the rate of microbial uptake (Table 6-5).

6.4 Discussion

Previous incubation studies have shown differences in CO_2 evolution from the same soil treated with different amendments (Söderstrom *et al.* 1983). So far, no explanation describes such differences and neither physical or chemical soil properties explain them (Agarwal *et al.* 1972). The present kinetic model addressing this question involves two microbial pools which were associated with microbial cytoplasmic and protein components (Chapter 4). The model helped to describe differences in CO_2 evolution between soils with different pedogenic and management histories (Chapter 5). The physiologic designation of pools has been accepted in this work to describe the dynamics of cystine through biontic components and to explain the differences in $^{14}CO_2$ respiration from amended soil samples.

6.4.1 Effect of amendments on the cycling of cystine-C in a Black Chernozemic soil

Earlier studies have shown diverse effects of various amendments on soil microbial processes. Addition of N or P sources stimulate CO_2 respiration (Roberge and Knowles, 1967; Van Cleve and Moore, 1978). On the other hand the addition of N to a Black cultivated and a Gray Luvisolic soil decreased the decomposition of straw, alfalfa and glucose carbon (Leuken *et al.* 1962). Also, N additions to a native Brown soil decreased the annual CO_2 output from the soil (De Jong *et al.* 1974). In the present experiments all amendments increased the $^{14}CO_2$

respiration from cystine in the Black soil. The kinetic model of Figure 6-1B describes the experimental observations obtained from the Black Chernozemic soil. The model indicates most ^{14}C-cystine is adsorbed or evolved as CO_2. On average, addition of amendments increased the amount adsorbed from 25 to 60% after six hours of incubation. Conversely, the amount of ^{14}C incorporated into cell proteins decreased from 45% to < 12 in samples treated with N, P, S, NPS, and to $< 2\%$ in samples pretreated with barley straw and alfalfa hay (Figure 6-4). Efficiency of substrate utilization by soil microbial biomass was affected by amendments. Microbial-^{14}C was simulated for 6 hours. The microbial-^{14}C content ranged between 8.7 and 13.8% of the initially added dose in samples amended with N,P,S,NPS, glucose and amino acid. The efficiency of substrate utilization decreased from 65% in control samples (Chapter 5) to about 25% in samples amended with glucose, amino acid mixture, N,P,S, NPS and to $< 2\%$ in samples pretreated with alfalfa hay and barley straw. These striking differences in utilization efficiencies must be related to metabolic controls on C flows. Two metabolite components associated with microbial cytoplasmic amino acid and proteins control cystine dynamics in steady-state non allophanic soils (Chapter 3,4,5). The present kinetic model showed the half-life for the protein component changed from 58 hours in the control soil to 12 and 4 minutes in the straw and alfalfa pretreated samples, respectively. Both prokaryotic and eukaryotic cells present intracellular compartmentalized proteins where long-lived proteins coexist with short-lived proteins (Alberghina and Martegani, 1977). For example, the β-galactosidase half-lives range between a few minutes to more than 20 hours, depending on the nature of the terminal amino acid of the protein (Bachmair et al., 1986). Both, the change in protein half-lives and the low efficiency of substrate utilization show that most ^{14}C-cystine flows via protein catabolism in amended Black Chernozemic samples. Degradation of proteins is a function of the cell's physiological state and appears to be controlled differentially for individual proteins (Pontremoli and Melloni, 1986). It is hypothesized that added plant residues released soluble-C which created temporary conditions of microbial growth. These substrates and other nutrients were already depleted from solution at the time of ^{14}C-cystine addition. This adverse growth environment created conditions of microbial starvation and death. Under such conditions, a low energy cell charge activates catabolic enzymatic reactions (Atlas, 1986). Conversely, a condition of high cell energy charge stimulates biosynthetic reactions. Under such conditions C is allocated intracellularly into macromolecules and is not respired (Chapter 4). The model output is consistent with the latter hypothesis. Further, it shows that about 90% of $^{14}CO_2$ evolves from the short-lived protein pool in amended samples. In comparison, most ^{14}C oxidized to CO_2 in the control samples originate from the cytoplasmic pool (Figure 6-5).

6.4.2 Effect of amendments on the cycling of cystine-C in an Andept soil

Addition of plant residues did not affect $^{14}CO_2$ evolution from the Andept soil but changed cystine dynamics. The kinetic model of Figure 6-1B is consistent with the experimental results and further suggests a physiological control by two biontic pools. In soils, residues such as straw serve as substrates as well as living habitats for microorganisms (McGill *et al.* 1981). It is hypothesized that addition of plant residues to the Andept samples transferred the microbial activities from the organoallophane-solution interface to centres where straw and alfalfa reside such that cystine cycling in Andept samples treated with plant residues approached that observed in non-allophanic soils. This is supported by parameter values given by the kinetic model. About 42 and 31% of the initially added ^{14}C was incorporated into proteins and adsorbed after six hours, respectively (Figure 6-6A). The model also indicates that $>$ 95% of the $^{14}CO_2$ originated from the cytoplasmic pool which in turn contained $<$ 1% of the initially added cystine. Turnover rates for the cytoplasmic and protein pools are similar to those of the unamended Black Chernozemic soil. The half-life value for proteins in the Andept soil increased from 4.6 h in the control to 58 hours in samples amended with alfalfa. The slower protein turnover shows that cystine-C flows via anabolic reactions in the Andept soil samples pretreated with plant residues. Conversely, this growth *proviso* could not have existed in the Chernozemic samples at the time of cystine addition. A measurement of the adenylate energy charge for both soils can test the validity of this hypothesis. A slower microbial growth and adaptations to soil internal changes after inclusion of plant residues in the Andept may explain this difference. A slower N mineralization than in non-allophanic soils has been reported for this Andept soil (Monreal *et al.* 1981). Nitrogen and P inhibit microbial respiration (Kowalenko *et al.* 1978; Söderstrom *et al.* 1983) or do not affect it (Ino and Monsi, 1964). In perturbation studies, additions of > 400 μg P $g^{-1}$to Andepts of Columbia increased CO_2 evolution (Munevar and Wollum, 1977). On the other hand, N applications either depress or stimulate microbial respiration in volcanic soils (Jackman, 1960). In the present studies, the addition of the amino acid mixture, or of N, P, and S singly to the Andept reduced the amounts of ^{14}C oxidized to CO_2. The model attributes this reduction to a lower rate of microbial uptake and to stabilization of cystine by the active surfaces of the organo-allophane complex (Figure 6-6B). Borie and Zunino (1983) attributed the increase of organic C and P in Andepts of Chile to stabilization reactions of inorganic P with allophane or through associations of organic matter with inorganic P.

In spite of a stabilization reaction, addition of glucose and NPS did not affect the oxidation of cystine to CO_2. Energy or C sources such as glucose have been found to be limiting the microbial activity in the Andept soil (Monreal *et al.* 1981).

In conclusion, kinetic analysis and modelling helped to explain differences in $^{14}CO_2$ evolution from samples incubated with ^{14}C-cystine and amended with organic and inorganic substrates. The kinetic model attributes an increase in cystine respiration from Black Chernozemic samples to catabolism of C allocated into proteins with half-lives of a few minutes. Differences in $^{14}CO_2$ respiration from amended Black Chernozemic samples are associated with differences in turnover rates of proteins. Addition of plant residues to Andept soil samples causes microbial activity to approach that of Chernozemic soils. Cystine cycling is then physiologically controlled by the biontic pools of non-allophanic soils. Inhibition of cystine-C respiration by N, P, S, NSP and a mixture of aminoacids is attributed to a slower cycling of solution cystine and to stabilization reactions between the substrate and the active surfaces of the organoallophanic complex. The kinetic treatment of experimental data provided insights into the basic components controlling the cycling of cystine in amended allophanic and non-allophanic soils. The model also established a hypothesis relating cystine metabolism to short and long-lived microbial proteins.

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Table 6-1. Effect of amendments on the ^{14}C remaining in solution and evolved as CO_2 from Black Chernozemic and Andept soil samples during six hours.

		^{14}C (% of initial dose)								
Soil	Pool	Control	Straw	Alfalfa	Glucose	Amino Acid	N	P	S	NPS
Black Chernozemic (Cultivated)	Solution	0.04 a	0.05 a	0.04 a	0.02 a	0.03 a	0.03 a	0.04 a	0.04 a	0.05 a
	CO_2	24.3 e	36.5 ab	39.4 a	28.5 d	31.6 c	31.7 c	30.0 cd	36.0 b	35.7 b
Andept (Cultivated)	Solution	0.03 a	0.16 a	0.24 a	0.06 a	0.06 a	0.03 a	0.09 a	0.08 a	0.08 a
	CO_2	21.6 ab	22.5 ab	23.4 a	21.1 ab	13.9 c	15.1 c	14.8 c	14.6 c	20.1 b

[1] Within rows, values not followed by the same letter are significantly different from each other at $p = 0.05$.

Table 6-2. The dynamics of ^{14}C-cystine in a Black Chernozemic soil pretreated with alfalfa and described by models of Figure 6-1A and 6-1B.

	^{14}C (% of initial dose)					
	Soil Solution			CO_2		
		Predicted			Predicted	
Time (min)	Observed	Figure 6-1a	Figure 6-1b	Observed	Figure 6-1a	Figure 6-1b
0	100.0000	99.9990	99.9990	0.00	0.00	0.00
5	0.5814	0.5767	0.3000	8.29	7.24	6.36
15	0.1365	0.1392	0.0912	21.06	16.47	21.60
30	0.0797	0.0891	0.0311	25.43	24.51	28.77
60	0.0546	0.0521	0.0289	30.03	32.43	30.69
120	0.0479	0.0436	0.0271	34.00	40.37	32.61
180	0.0457	0.0418	0.0254	35.88	47.51	34.46
240	0.0435	0.0405	0.0237	37.99	52.25	36.27
360	0.0412	0.0398	0.0215	39.36	56.83	39.71
$(x^2)^1$		0.003	0.45		14.76	1.20

[1] $(CHI)^2 = 3.84$ at $p = 0.05$ with 1 degree of freedom.

Table 6-3. The dynamics of ^{14}C-cystine in an Andept soil pretreated with alfalfa and described by models of Figure 6-1A and 6-1B.

Time (min)	^{14}C (% of initial dose)					
	Soil Solution			CO_2		
		Predicted			Predicted	
	Observed	Figure 6-1a	Figure 6-1b	Observed	Figure 6-1a	Figure 6-1b
0	100.00	100.00	100.00	0.00	0.00	0.00
5	0.8	0.79	0.61	1.93	2.17	3.23
15	0.48	0.46	0.59	3.89	6.42	5.60
30	0.38	0.39	0.57	8.47	10.15	7.18
60	0.34	0.33	0.52	12.67	16.25	10.56
120	0.30	0.31	0.44	16.58	24.31	14.91
180	0.26	0.27	0.37	18.02	31.47	17.81
240	0.25	0.26	0.31	21.25	37.23	21.17
360	0.24	0.24	0.22	23.38	42.04	24.74
(x^2)[1]		0.003	0.30		26.58	1.38

[1] $(CHI)^2 = 3.84$ at $p = 0.05$ with 1 degree of freedom.

Table 6-4. The dynamics of ^{14}C-cystine in a P amended Andept soil described by the models of Figure 6-1B and 6-1C.

	^{14}C (% of initial dose)					
	Soil Solution			CO_2		
		Predicted			Predicted	
Time (min)	Observed	Figure 5-1c	Figure 5-1b	Observed	Figure 5-1c	Figure 5-1b
0	100.0000	99.9990	99.9990	0.00	0.00	0.00
5	0.1563	0.4388	3.5453	0.32	0.44	0.29
15	0.1196	0.4147	3.1787	1.60	1.40	1.20
30	0.1145	0.3966	2.6997	2.47	2.80	2.75
60	0.1082	0.3291	1.9462	5.32	5.37	5.53
90	0.1048	0.2658	1.4021	7.97	7.48	7.65
120	0.0939	0.2151	1.0111	8.90	9.17	9.25
180	0.0899	0.1433	0.5284	11.94	11.53	11.43
240	0.0876	0.0912	0.2733	12.50	12.98	12.81
360	0.0859	0.0690	0.0738	14.53	14.40	14.53
$(x^2)^1$		3.57	32.46		0.17	0.13

[1] $(CHI)^2 = 3.84$ at $p = 0.05$ with 1 degree of freedom.

Table 6-5. Rate constants (k, min^{-1}) for cystine cycling through amended Black Chernozemic and Andept soils described by models of Figures 6-1.

	Black Chernozemic[1]					Andept[3]			
	Control	Alfalfa	Straw[2]	Glucose	Amino acid	Control[4]	Alfalfa[5]	P[6]	Amino acid
	Turnover rate (min^{-1})								
Compartment									
Soil solution	3.0	3.8	3.6	3.7	3.8	8.4	3.2	6.7	5.7
Cytoplasmic cystine	0.76	0.17	0.17	0.35	0.16	N/A	0.55	N/A	N/A
Adsorbed	0.017	0.002	0.002	0.002	0.002	0.009	0.023	0.036	0.056
Protein	0.0002	0.182	0.058	0.003	0.006	0.0025	0.0002	0.036	0.005
Stabilized	N/A[7]	N/A	N/A	N/A	N/A	0	N/A	0	0
	Rate constant (min^{-1})								
Reaction									
Adsorption (k_1)	2.4	2.7	2.7	2.4	2.7	6.5	2.8	6.5	5.5
Desorption (k_2)	0.017	0.002	0.002	0.0016	0.002	0.008	0.023	0.03	0.05
Uptake (k_3)	0.58	1.11	0.90	1.30	1.11	1.87	0.40	0.19	0.17
Protein synthesis (k_4)	0.48	0.16	0.16	0.26	0.16	N/A	0.35	N/A	N/A
Cytoplasm-C oxid. (k_5)	0.28	0.008	0.008	0.09	0.002	N/A	0.198	N/A	N/A
Protein-C oxid. (k_6)	0.0002	0.182	0.058	0.003	0.006	0.0075	0.0002	0.036	0.005
Stabilization (k_7)	N/A	N/A	N/A	N/A	N/A	0.001	N/A	0.006	0.006

[1] The four compartment model described in Figure 6-1b was accepted for the Black amended samples.
[2] Straw amended samples were representative of the cystine dynamics in samples treated with N,P,S and NPS.
[3] Scenarios of Figures 6-1B,C describe the cycling of cystine-C in the Andept.
[4] Assuming that cystine-C cycles through the system of Figure 6-1C which includes a stabilized pool.
[5] Cystine-C cycles through the four components of Figure 6-1B in the alfalfa and straw pretreated samples.
[6] The P treatment illustrates the effects of N, S.
[7] N/A: Pool or reaction not defined for that specific model.

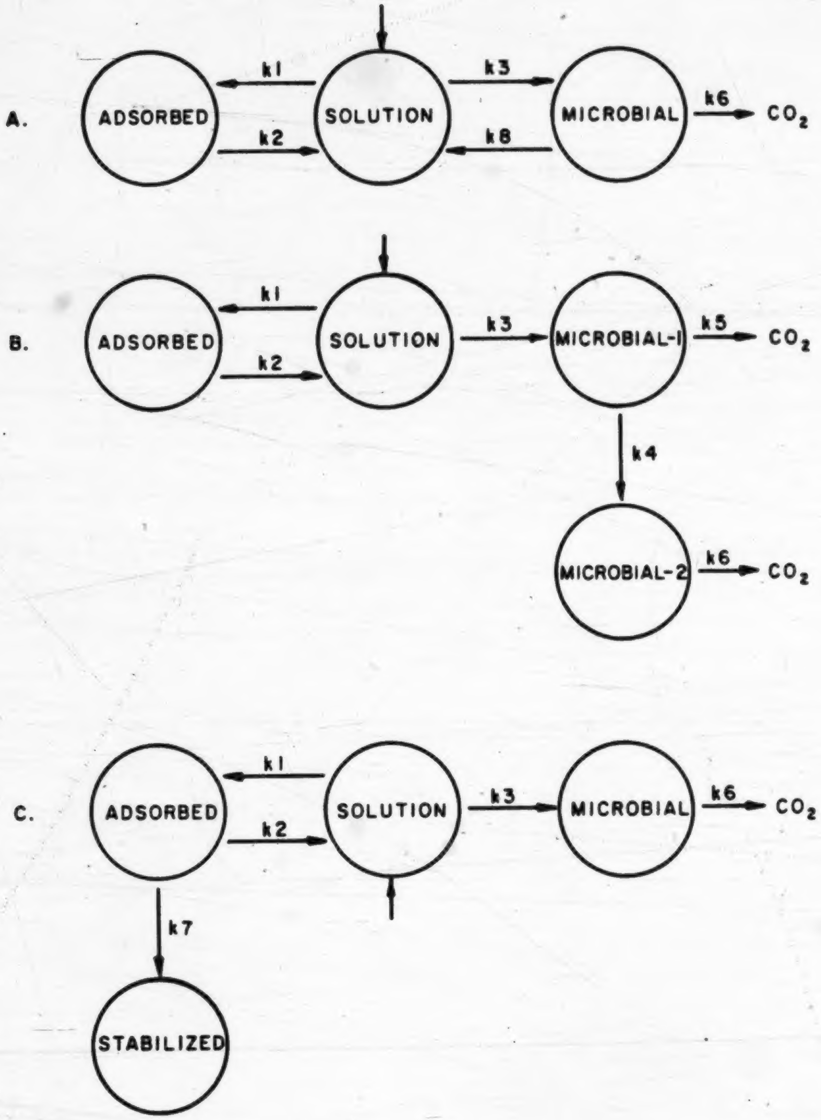
A.
ADSORBED
SOLUTION
MICROBIAL
k1
k2
k3
k8
k6
CO2
B.
ADSORBED
SOLUTION
MICROBIAL-1
MICROBIAL-2
k1
k2
k3
k4
k5
k6
CO2
CO2
C.
ADSORBED
SOLUTION
MICROBIAL
STABILIZED
k1
k2
k3
k6
k7
CO2

Figure 6-1. Three hypotheses representing the cycling of free cystine through various components in treated Black Chernozemic and Andept soil ecosystems.

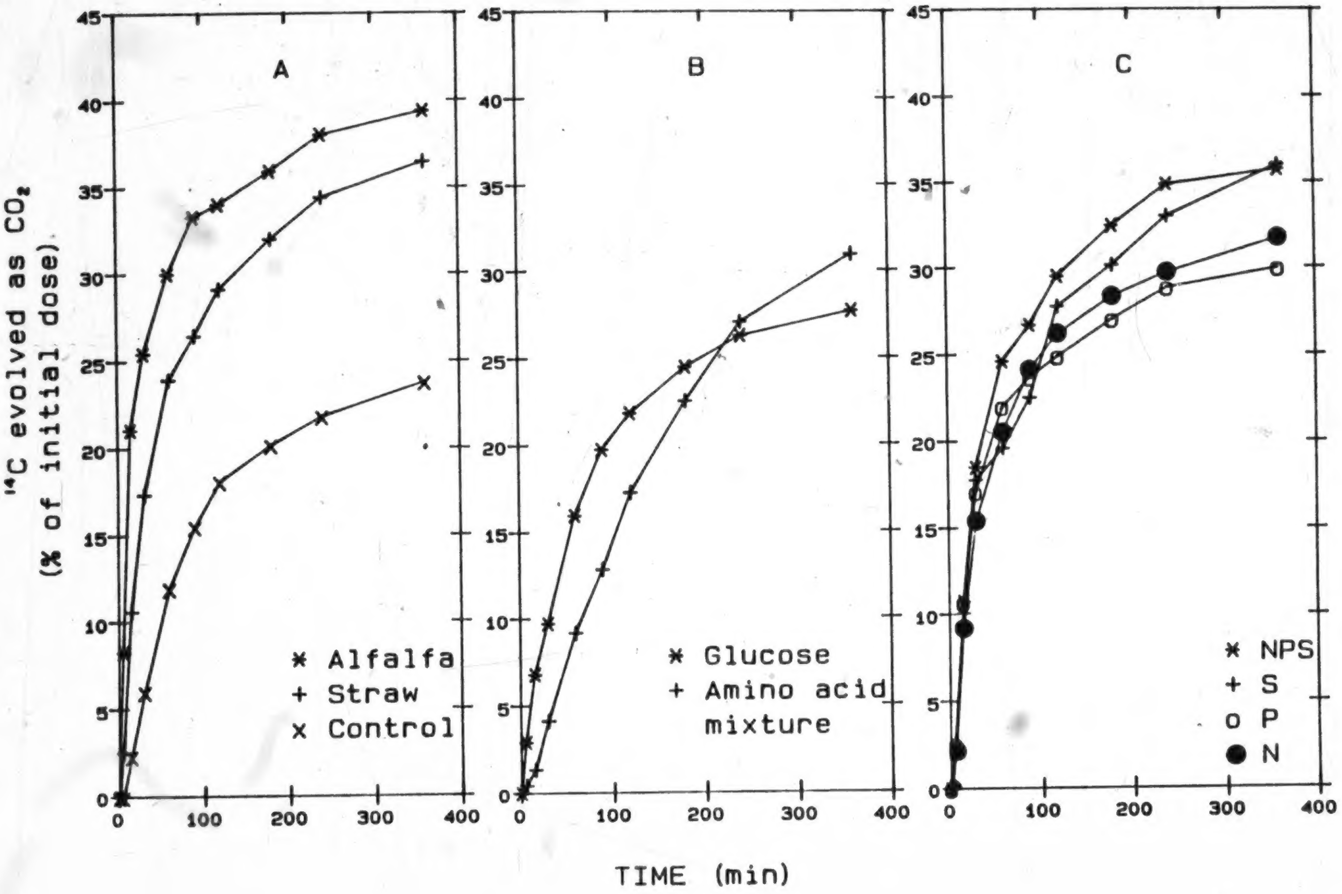

Figure 6-2. Oxidation of ^{14}C-cystine to CO$_2$ in amended Black Chernozemic soil samples.

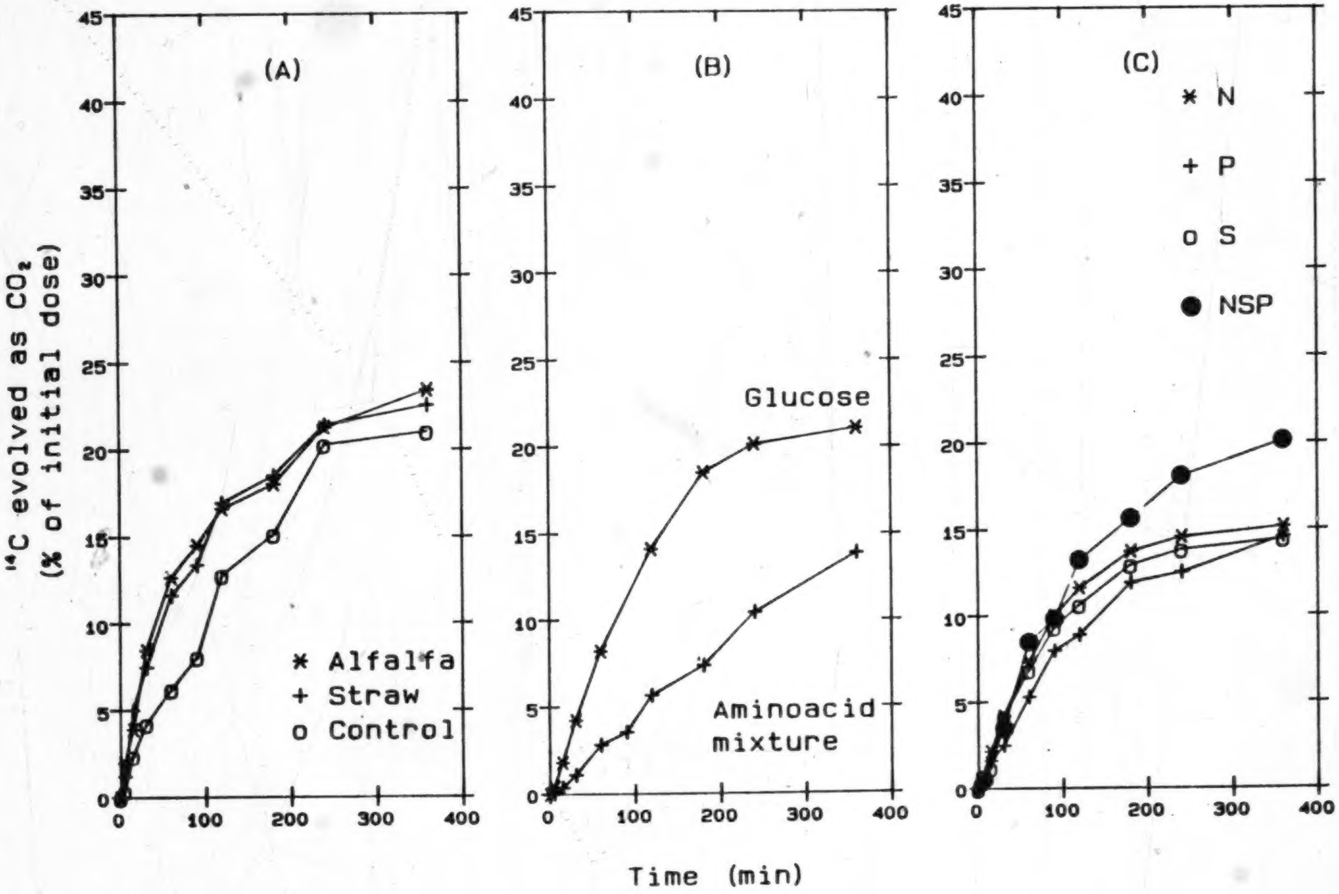

Figure 6-3. Oxidation of ^{14}C-cystine to CO_2 in amended Andept soil samples.

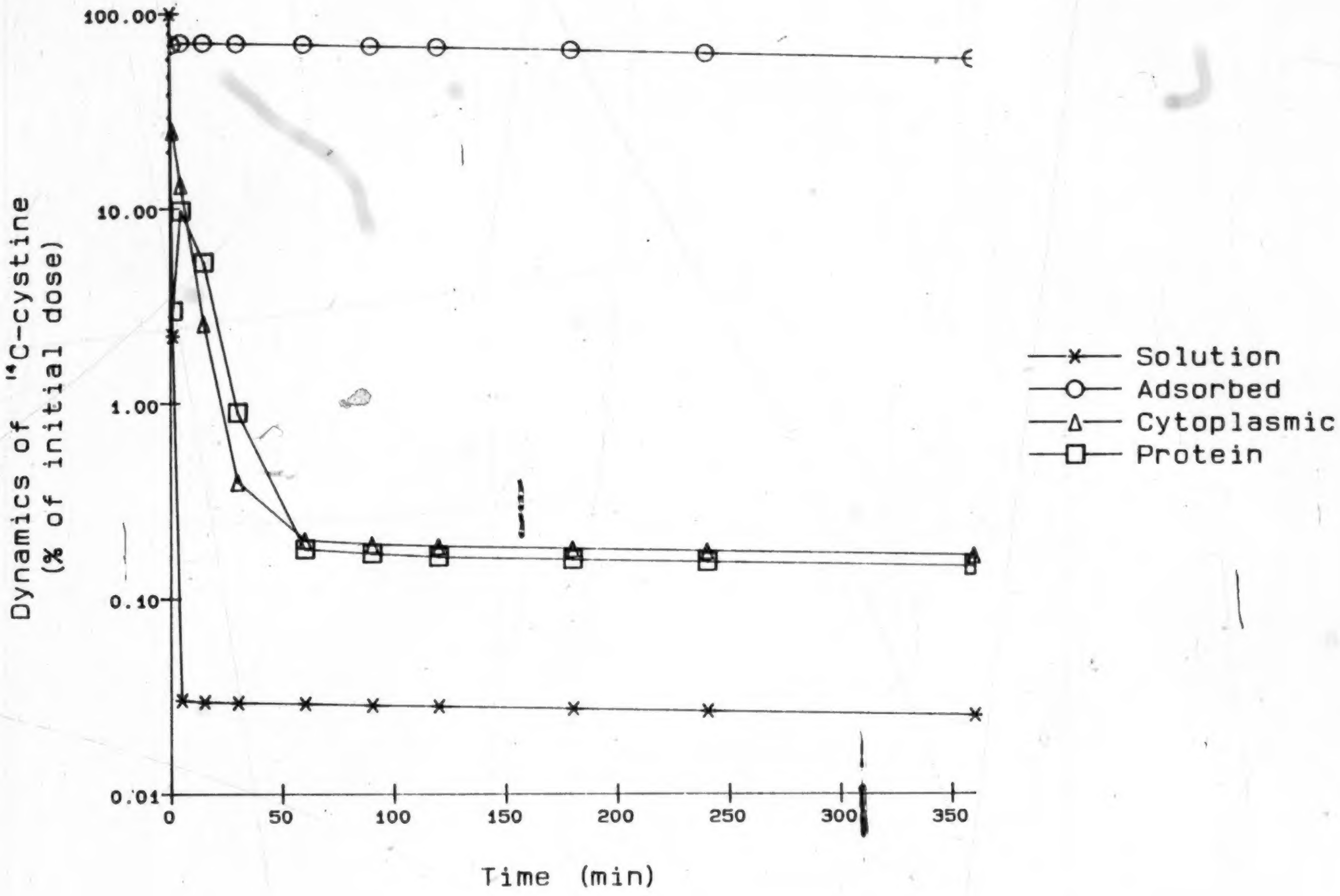

Figure 6-4. Dynamics of ^{14}C-cystine cycling through four components in Black Chernozemic samples pretreated with alfalfa hay. This example illustrates the effects caused by additions of N, P, S, straw, glucose and a mixture of amino acids.

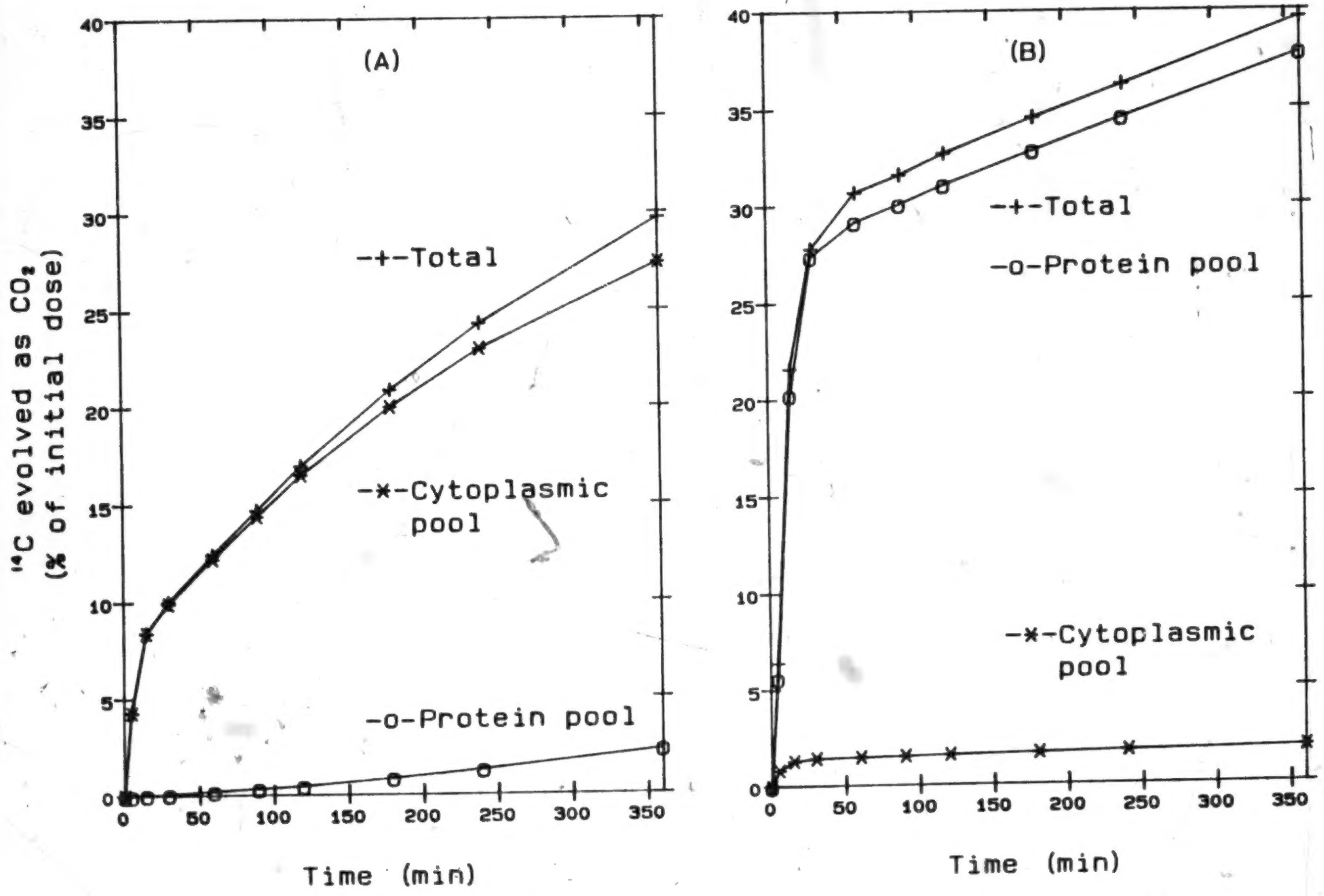

Figure 6-5. Predicted sources of $^{14}CO_2$ evolved from amended Black Chernozemic samples. A=control, B=alfalfa.

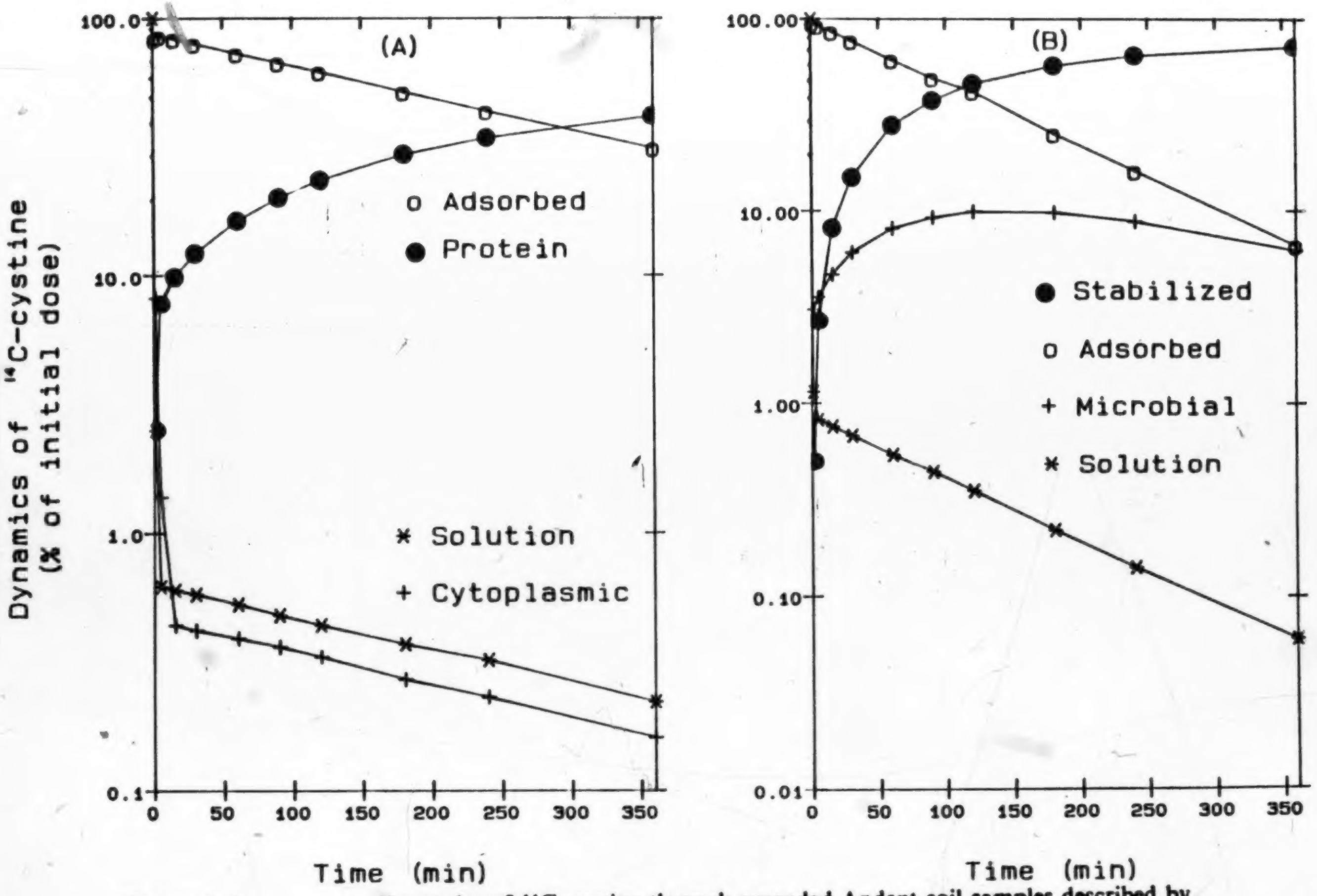

Figure 6-6. Dynamics of ^{14}C-cystine through amended Andept soil samples described by models of Figure 6-1b and 6-1c. (A) Samples pretreated with alfalfa hay and barley straw. (B) Samples amended with a mixture of amino acids, glucose, N, P, S and NPS.

7. SYNTHESIS

Rapid reactions influence the flows of energy and nutrients from soil soluble fractions. Experimental observations of soil organic matter changes over long periods (days, weeks) mask effects of fast processes occurring over short periods (minutes, hours). Soil organic matter dynamics have been simulated for processes on a daily time step (McGill *et al.* 1981; van Veen *et al.* 1985). These models do not have the resolution to describe fast processes affecting soluble-C fractions over short periods.

Abiotic and biontic components controlling soluble-C dynamics were studied through short term incubation experiments. Kinetic analysis and modelling were used as tools to study the control mechanisms of: a)cystine dynamics in non-perturbed soils and b) glucose-C dynamics in perturbed soils. Glucose and cystine were chosen as substrates because they are important constituents of soil organic matter (Bremner, 1949). A necessary condition to study control mechanisms *in vivo* in steady-state soil systems is to use molecules found "in situ" and at low concentration to minimize system distortions. Under non-perturbed conditions specific and high affinity enzymatic systems transport substrates very rapidly across microbial cell membranes (Anraku, 1980). Within this context, studies were conducted to characterize the free amino acid fraction prior to studying their dynamics in soils at steady-state (Chapter 1). One allophanic and eight non-allophanic soils with different management histories contained diverse and low contents of such molecules.

Experiments to test conceptual models describing soluble-C dynamics used cystine at low concentrations to maintain the steady-state of soils (Chapter 3). The basic model components were separated into kinetically homogeneous abiotic and biontic parts comprising the soil solution, adsorbed phase and two microbial pools metabolizing C intracellularly (Chapter 3). The kinetic model simulated outputs were consistent with experimental observations; showing that the low content in and dynamics of cystine-C through soil solutions was controlled by microbial metabolism in non-allophanic soils, and by microbial metabolism and abiotic stabilization in the allophanic soil. Adsorption did not prevent microbial use of cystine (Chapter 3).

Behaviour of the two biontic components controlling cystine cycling at steady-state was analogous to microbial cytoplasmic amino acids and intracellular proteins (Chapter 4). In addition, the plateau in $^{14}CO_2$ evolution attained over six hours in all soils studied (Chapter 3,4,5) is interpreted as microbial stabilization of substrate-C, behaving as if allocated into long-lived proteins which are slowly degraded.

Within microbial cells, cystine can be metabolized via catabolism or anabolism depending on the growth media (Beilan et al., 1983). The conversion of cystine to cysteine and pyruvate may serve to generate energy, to synthesize other intermediates of metabolism or to synthesize alanine, valine, and leucine for incorporation into proteins.

During ATP synthesis, pyruvate is oxidized in the Krebs cycle to CO_2 and generation of reducing power (NADH). Oxidative phosphorylation reoxidizes the nucleotide at the membrane with a concomitant production of ATP (Atlas,1986).

Catabolism reduces cystine to cysteine with utilization of diphosphopryridine nucleotide (DPNH). *Escherichia coli* decomposes cysteine to pyruvate by known pathways. The basic steps include desulfhydration and deamination enzymatic reactions (Meister, 1965). Key intermediates in these catabolic reactions include β-mercaptopyruvate, cysteinesulfinate, alanine and β-sulfinylpyruvate (Figure 1, Appendix). Anaerobic decomposition of cysteine results in the formation of pyruvate, H_2S and NH_3 (Meister, 1965).

Intact cysteine molecules can be incorporated into proteins by *Escherichia coli* cells growing in a media containing all 20 amino acids (Beilan et al., 1983). This reaction is catalyzed by aminoacyl synthase and requires energy in the form of guanosine triphosphate. Proteins are assembled at the ribosomes and involve tRNA and mRNA (Atlas, 1986).

A modified model was required to describe C dynamics in disturbed soil systems. Under such conditions, glucose-C is best described as being allocated into intermediates of metabolism and into non-dagrading macromolecules such as proteins or RNA (Chapter 4). In both models, pools of intermediates and macromolecules of metabolism describe the flows of soluble-C through soil organisms over short periods of time under both, perturbed and non-perturbed conditions. This treats microbial biomass as the main catalyst in soil organic matter transformations, and suggests that microbial stabilization of soluble-C fractions is quantitatively more important than abiotic stabilization reactions over short periods in non-allophanic soils. The kinetic models of cystine dynamics are consistent with physiologic rather than taxonomic controls on internal cycling rates. The models could not separate prokaryiotic and eukaryiotic activities. This implies that metabolic reaction rates are the expression of only one microbial group or that both taxa have similar reaction rates to oxidize soluble-C in soils.

The physiologic controls on cystine dynamics are also expressed in soils with different pedogenic histories, management and agronomic practices. So far, different CO_2 respiration patterns from disturbed soil samples have not been explained and much published information only speculates on possible causes (Söderstrom *et al.* 1983). In this thesis, perturbation of soil systems with cultivation affected the metabolic source of respired carbon. The model shows that the fast cycling cytoplasmic pool is the main source of $^{14}CO_2$ evolved from cultivated samples. Conversely, $^{14}CO_2$ respired from virgin soils originated mainly from cell proteins. Estimates of pool sizes show up to 6 times more cystine-C is stored by microbial cells in virgin than in cultivated samples (Chapter 5). The larger $^{14}CO_2$ respiration from cultivated samples implies a lower carbon utilization efficiency. The efficiency of ^{14}C utilization averaged 76% in virgin soils and 60% in cultivated soils (Chapter 5). The lower efficiency in cultivated samples can be related to lower microbial biomass (Voroney *et al.* 1981), or to soil solution composition.

Perturbations such as addition of amendments to allophanic and non allophanic soils decreased the amounts of substrate-C retained within microbial cells. The changes in solution composition with chemical amendments and of internal soil architecture with plant residues altered cystine metabolism. Under the exclusion of all amendments most cystine-C was used via anabolic processes to build long-lived proteins. Conversely, addition of all amendments (except straw and alfalfa in the Andept) caused most cystine-C to be allocated into short-lived proteins or catabolized to produce energy (Chapter 6). The addition of all amendments decreased the ^{14}C-cystine utilization efficiency by at least 20%. This shift in dynamics must be associated with controls of microbial metabolism. That is, types of enzymes, allosteric effectors, or the cell energy charge were altered by such disturbances. If cystine was catabolized for ATP synthesis, then cells in amended soils must present a lower energy charge than microorganisms living in unamended soils.

Other tests for both models should be based on the above molecular level information and may include the following determinations in soil samples supplemented with ^{14}C labeled substrates:

a) Determine the proportion of substrate-C evolved as CO_2 by using ^{14}C-carboxylic labeled cystine. This would directly estimate catabolic reaction rates and indirectly estimate the proportion of substrate-C used in anabolic reactions.

b) Determine the ratio of reduced nicotineamide adenine dinucleotide (NADH)/nicotineamide dinucletide phosphate (NADP) as an index for the direction of C flow. NADH production is coupled to catabolic reactions during ATP synthesis. NADP generation is coupled to anabolic pathways during the synthesis of macromolecules.

c) Determine ^{14}C activity recovered in soil extracted RNA and/or DNA.

d) Determine aminoacyl synthase activity for aminoacid incorporation into proteins.

e) Determine ^{14}C content in microbial proteins.

Differences between the allophanic and non-allophanic soils are reflected in one abiotic stabilizing reaction and one single physiological pool in the Andept soil (Chapter 3). It is hypothesized the single microbial pool of the Andept soil can either exist as proteins in microbial cells devoid of cytoplasmic free amino acids or be related to a single taxa of microorganisms using cystine. Zunino et al. (1982) reported a predominance of fungi and actinomycetes in Andepts of Chile. Abiotic adsorption and stabilization reactions have been documented for allophanic and non-allophanic soils (Espinoza *et al.* 1975; Borie and Zunino, 1983; Harter, 1977).

In these experiments adsorption did not prevent microbial utilization of cystine in either allophanic or non-allophanic soils. Nevertheless, Dashman and Stotsky (1986) indicated that adsorption reactions precluded microbial use of cysteine in a pure clay-cysteine-microbial system. Differences in substrate utilization capacities and internal architecture between biontic and abiotic components or microbial adaptations *in vivo* soil ecosystems may explain these differences.

Results from the present investigations provide new information to soil chemistry. Classical soil chemical equilibria and adsorption studies do not consider effects of soil microorganisms. The kinetic model separates concurrent reactions of adsorption and desorption, calculates their rates and estimates the "equilibrium" concentration for cystine-C in the presence of active microorganisms. For example, applications from such models or similar approaches may have implications to mineral weathering processes and fertilizer recommendations.

Stabilization of cystine to organoallophanic surfaces was of minor importance in sytems where plant residues changed the internal architecture of soils. Under such conditions, cystine dynamics was best described by two biontic pools similar to those existing in non-allophanic soils (Chapter 6). If the model representation is correct, the shift in microbial activities in the allophanic soil has to be related to a different microbial community.

At present, it is unknown if the active cells using soluble-C sources under both, perturbed and non-perturbed conditions are prokaryotes, eukaryotes or both. Published data indicate that bacteria are the main group using soluble substrates in aquatic systems (Hobbie and Crawford, 1969) and in other environments (Mandelstam and McQuillen, 1973). The present experimental data and kinetic modelling appear not to reflect the activities of two microbial taxa. At present, analytical tecniques and model resolution do not permit a more rigorous test of this alternative hypothesis. This unresolved issue needs to be addressed by future research in the fields of soil microbiology and biochemistry.

In summary, kinetic analyses and modelling of *in vivo* soil systems assisted in describing dynamics of soluble organic molecules. Short-term cycling of soluble organic molecules under both perturbed and non-perturbed conditions in the laboratory is best described by models incorporating microbial metabolism of cytoplasmic materials and macromolecules such as proteins. In allophanic and non-allophanic soils disturbed with organic and inorganic amendments the controls are physiological and abiotic. The physiologic controls are applicable to soils with a wide range of properties and for diverse conditions of microbial growth, soil pedogenic processes and management histories. The broad representation of soil systems by such models reafirms their generality. The kinetic approach used in these studies represent a significant contribution to the areas of soil biology and biochemistry. Systems like the present kinetic model may clarify some of the contradictions and confusion concerning processes affecting organic matter transformations (Stotsky, 1986). The models supplement existing knowledge and provide new elements for a better understanding of mechanisms controlling the release of nutrients from soil organic matter for plant growth. These kinetic models point to areas in need of further research.

7.1 CONCLUSIONS

The description of cystine cycling by kinetic analysis permit the following conclusions:

1. Four soil components describe the cycling of cystine in non-perturbed Chernozemic and Luvisolic soils. These are the soil solution, adsorbed, microbial cytoplasm cystine and proteins. For allophanic soils the system is best represented by solution, adsorbed, stabilized and one biontic pool similar to the protein pool of the non-allophanic soils.
2. Reactions transferring cystine in Chernozemic and Luvisolic soils are adsorption, desorption, microbial uptake, protein synthesis and oxidation to CO_2 from cytoplasmic cystine and intracellular proteins. In addition to the former reactions, an abiotic stabilization reaction and respiration from microbial proteins characterize the Andept soil.

3. The fastest cycling pool is the soil solution (seconds) followed by adsorbed and cytoplasmic cystine (minutes) and cell protein-C (hours). In the Andept soil, the stabilized-C does not turnover during the period of study.
4. Greater $^{14}CO_2$ respiration from native Brown than from Dark Brown and Black Chernozemic soils is associated with a faster turnover of solution cystine in the former soil.
5. Differences in CO_2 evolution from cultivated and virgin samples are attributed to the source of CO_2. In cultivated samples most ^{14}C is respired from the cytoplasmic pool; and in virgin samples from cell proteins. Six times more substrate-C is found in the active cells of virgin soils.
6. Incorporation of plant residues shifts the center of microbial activities to the surface of decomposing alfalfa and grain straw residues. Under such conditions, cystine dynamics in allophanic and non-allophanic soils converge.
7. Addition of most organic and inorganic amendments decreased the amount of cystine-C incorporated into microbial cells. The amounts adsorbed and stabilized increased and cell proteins cycled faster.
8. Increases in $^{14}CO_2$ evolution from amended Black Chernozemic samples are associated with degradation of cystine-C incorporated into short-lived proteins.
9. Inhibition in $^{14}CO_2$ evolution from Andept samples is attributed to an increase in stabilization of cystine with the active organoallophanic surfaces.
10. Two biontic components control the flow of glucose-C in perturbed soil systems. The two components are intermediates of metabolism and stable macromolecules such as proteins, RNA or DNA.
11. Kinetic analysis and modelling techniques assist in describing soil components and reactions affecting the cycling of organic molecules under perturbed and non-perturbed conditions. The kinetic models suggest that controls on the internal cycling of soluble organic molecules are physiological rather than abiotic during short term incubations.

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8. APPENDIX

Table 1. The ^{14}C remaining in solution and evolved as CO_2 in unamended and amended soil samples. Values are expressed as % of initial dose (q_{so}) ± standard deviation of sample replicates. Symbols represented by V = virgin, C = cultivated, N=Nitrogen, S=Sulphur and P=Phosphorus, dpm=disintegration per minute.

Time min	Dark Brown - V		Dark Brown - C	
	Solution	CO_2	Solution	CO_2
0	100.0000	0.0	100.0000	0.0
1	1.6465 ± 0.131	—	2.6873 ± 0.252	—
5	0.3008 ± 0.012	0.1 ± 0.10	0.5399 ± 0.042	1.7 ± 0.13
15	0.0873 ± 0.002	0.5 ± 0.09	0.2460 ± 0.028	4.5 ± 0.21
30	0.0580 ± 0.003	1.2 ± 0.47	0.1635 ± 0.028	8.4 ± 0.08
60	0.0498 ± 0.003	2.8 ± 0.66	0.1383 ± 0.018	10.7 ± 0.14
90	0.0457 ± 0.004	5.7 ± 0.70	0.1276 ± 0.014	13.8 ± 0.06
120	0.0428 ± 0.003	7.7 ± 0.07	0.1230 ± 0.016	15.7 ± 0.11
180	0.0404 ± 0.000	11.3 ± 0.09	0.1190 ± 0.015	18.9 ± 0.33
240	0.0387 ± 0.001	15.9 ± 0.12	0.1157 ± 0.001	21.0 ± 0.61
360	0.0369 ± 0.002	19.8 ± 0.34	0.1137 ± 0.001	24.3 ± 0.89
qso	170538 dpm		150372 dpm	
	Brown - V		Brown - C	
0	100.0000	0.0	100.0000	0.0
1	0.8251 ± 0.093	—	2.8803 ± 0.307	—
5	0.2056 ± 0.021	0.6 ± 0.02	0.4892 ± 0.028	3.2 ± 0.07
15	0.1013 ± 0.028	3.4 ± 0.07	0.2297 ± 0.001	4.2 ± 0.29
35	0.0956 ± 0.014	8.4 ± 0.60	0.1595 ± 0.014	6.7 ± 0.30
60	0.0660 ± 0.002	11.0 ± 0.30	0.1373 ± 0.003	9.3 ± 0.10
90	0.0620 ± 0.003	13.6 ± 0.90	0.1305 ± 0.001	12.7 ± 0.06
120	0.0564 ± 0.001	15.4 ± 0.14	0.1236 ± 0.000	13.9 ± 0.34
180	0.0544 ± 0.000	17.8 ± 0.77	0.1152 ± 0.004	16.4 ± 0.47
240	0.0504 ± 0.001	19.4 ± 0.66	0.1114 ± 0.002	18.8 ± 0.94
360	0.0483 ± 0.002	21.1 ± 0.27	0.1068 ± 0.001	23.1 ± 0.13
qso	198379 dpm		161027 dpm	

(continued...)

Time min	Gray Luvisolic (2 year rotation)		Gray Luvisolic (5 year rotation)	
	Solution	CO_2	Solution	CO_2
0	100.0000	0.00	100.0000	0.0
1	1.7610 ± 0.170	—	2.0411 ± 0.175	—
5	0.3315 ± 0.042	1.4 ± 0.16	0.4517 ± 0.004	3.9 ± 0.42
15	0.1561 ± 0.016	4.4 ± 0.12	0.2160 ± 0.020	6.3 ± 0.21
30	0.1144 ± 0.009	10.6 ± 0.41	0.1605 ± 0.018	9.5 ± 0.53
60	0.0919 ± 0.019	14.6 ± 0.57	0.1370 ± 0.004	11.7 ± 0.39
90	0.0794 ± 0.010	16.1 ± 0.27	0.1292 ± 0.018	14.3 ± 0.31
120	0.0747 ± 0.003	19.0 ± 0.36	0.1227 ± 0.011	15.8 ± 0.17
180	0.0708 ± 0.002	21.4 ± 0.68	0.1174 ± 0.010	18.9 ± 0.10
240	0.0674 ± 0.001	25.8 ± 0.22	0.1142 ± 0.000	23.9 ± 0.57
360	0.0648 ± 0.003	29.6 ± 0.17	0.1109 ± 0.004	27.3 ± 0.67
qso	161115 dpm		163118 dpm	
	Black-C		Black - V	
0	100.0000	0.00	100.0000	0.0
1	1.7655 ± 0.010	—	—	—
5	0.6767 ± 0.014	2.3 ± 0.07	3.0500 ± 0.613	0.9 ± 0.06
15	0.3640 ± 0.014	6.0 ± 0.01	1.6894 ± 0.130	1.2 ± 0.00
35	0.2579 ± 0.042	11.9 ± 0.34	1.2012 ± 0.013	2.4 ± 0.02
60	0.2263 ± 0.049	15.5 ± 0.00	1.1103 ± 0.014	4.2 ± 0.30
90	0.2133 ± 0.028	18.1 ± 0.65	1.0245 ± 0.032	5.5 ± 0.51
120	0.1986 ± 0.029	20.2 ± 0.10	0.9417 ± 0.016	7.1 ± 0.12
180	0.1907 ± 0.015	21.8 ± 0.67	0.8891 ± 0.043	9.5 ± 0.24
240	0.1862 ± 0.009	23.8 ± 0.88	0.8349 ± 0.017	11.3 ± 0.45
360	0.1834 ± 0.001	24.3 ± 0.81	0.7946 ± 0.051	14.4 ± 0.57
qso	177168 dpm		178815 dpm	

(continued...)

Time (min)	Andept	
	Solution	CO_2
0	100.000	0.00
1	0.2100 ± 0.033	—
5	0.1558 ± 0.002	0.37 ± 0.04
17	0.0816 ± 0.006	1.23 ± 0.07
30	0.0616 ± 0.009	2.62 ± 0.06
60	0.0475 ± 0.002	5.22 ± 0.08
90	0.0441 ± 0.001	7.55 ± 0.10
120	0.0416 ± 0.001	10.21 ± 0.07
180	0.0400 ± 0.000	14.94 ± 0.03
240	0.0366 ± 0.001	19.42 ± 0.05
360	0.0333 ± 0.003	23.67 ± 0.03
qso	170000 dpm	

	Andept - alfalfa		Andept - Straw	
0	100.0000	0.00	100.0000	0.0000
1	1.7592 ± 0.010	—	1.1545 ± 0.027	—
5	0.8077 ± 0.070	1.93 ± 0.05	0.4476 ± 0.012	1.22 ± 0.01
15	0.4777 ± 0.012	3.89 ± 0.70	0.2652 ± 0.007	5.01 ± 0.12
30	0.3792 ± 0.022	8.47 ± 0.32	0.2105 ± 0.005	7.48 ± 0.68
60	0.3442 ± 0.037	12.67 ± 0.79	0.1901 ± 0.002	11.63 ± 0.13
90	0.3240 ± 0.009	14.52 ± 0.07	0.1862 ± 0.010	13.36 ± 0.27
120	0.3009 ± 0.008	16.58 ± 0.62	0.1774 ± 0.003	16.91 ± 0.34
180	0.2581 ± 0.010	18.02 ± 0.66	0.1702 ± 0.004	18.48 ± 0.06
240	0.2492 ± 0.009	21.25 ± 0.80	0.1691 ± 0.004	21.40 ± 0.74
360	0.2463 ± 0.003	23.38 ± 0.71	0.1613 ± 0.007	22.53 ± 0.14
qso	168488 dpm		180944 dpm	

(continued...)

Time min	Andept - N		Andept - P	
	Solution	CO_2	Solution	CO_2
0	100.0000	0.00	100.0000	0.00
5	0.0901 ± 0.007	0.45 ± 0.07	0.1563 ± 0.071	0.32 ± 0.03
15	0.0529 ± 0.003	2.12 ± 0.22	0.1196 ± 0.009	1.60 ± 0.01
30	0.0469 ±0.001	4.20 ± 0.69	0.1145 ± 0.008	2.47 ± 0.42
60	0.0419 ± 0.001	7.34 ± 0.54	0.1082 ± 0.003	5.32 ± 0.30
90	0.0409 ± 0.002	10.02 ± 0.06	0.1048 ± 0.004	7.97 ± 0.16
120	0.0398 ± 0.000	11.62 ± 0.31	0.0939 ± 0.005	8.90 ± 0.60
180	0.0383 ± 0.001	13.70 ± 0.31	0.0899 ± 0.002	11.94 ± 0.88
240	0.0362 ± 0.003	14.56 ± 0.08	0.0876 ± 0.006	12.50 ± 0.62
360	0.0346 ± 0.001	15.12 ± 0.18	0.0859 ± 0.001	14.53 ± 0.81
qso	190834 dpm		174677 dpm	
	Andept - S		Andept - NPS	
0	100.0000	0.00	100.0000	0.00
5	0.1484 ± 0.010	0.39 ± 0.06	0.1603 ± 0.008	0.52 ± 0.02
15	0.1185 ± 0.009	1.23 ± 0.27	—	1.77 ± 0.15
30	0.1121 ± 0.003	4.16 ± 0.37	0.1079 ± 0.009	3.47 ± 0.30
60	0.1050 ± 0.002	6.88 ± 0.25	0.1025 ± 0.001	8.45 ± 0.48
90	0.0945 ± 0.004	9.35 ± 0.42	0.0989 ± 0.003	9.82 ± 0.00
120	0.0904 ± 0.001	10.65 ± 0.23	0.0918 ± 0.007	13.23 ± 0.00
180	0.0863 ± 0.002	12.95 ± 0.69	0.0882 ± 0.003	15.62 ± 0.29
240	0.0845 ± 0.005	13.81 ± 0.44	0.0858 ± 0.005	18.03 ± 0.84
360	0.0827 ± 0.006	14.40 ± 0.67	0.0840 ± 0.002	20.10 ± 0.42
qso	170431 dpm		167818 dpm	
	Andept-Glucose		Andept-amino acid mixture	
0	100.0000	0.00	100.0000	0.00
5	0.1204 ± 0.023	0.38 ± 0.07	0.1534 ± 0.011	0.12 ± 0.01
15	0.0998 ± 0.007	1.80 ± 0.17	0.1030 ± 0.007	0.37 ± 0.00
30	0.0903 ± 0.005	4.20 ± 0.18	0.0892 ± 0.005	1.12 ± 0.05
60	0.0838 ± 0.003	8.21 ± 0.54	0.0796 ± 0.006	2.80 ± 0.16
90	0.0794 ± 0.002	11.84 ± 0.06	0.0749 ± 0.007	3.53 ± 0.33
120	0.0756 ± 0.007	14.13 ± 0.62	0.0696 ± 0.001	5.69 ± 0.84
180	0.0638 ± 0.001	18.47 ± 0.72	0.0658 ± 0.000	7.38 ± 0.88
240	0.0632 ± 0.003	20.14 ± 0.11	0.0611 ± 0.003	10.48 ± 0.30
360	0.0612 ± 0.003	21.06 ± 0.99	0.0595 ± 0.005	13.88 ± 0.57
qso	169408 dpm		188326 dpm	

(continued...)

Time min	Black - C.N		Black - C.P	
	Solution	CO$_2$	Solution	CO$_2$
0	100.0000	0.0000	100.0000	0.0000
1	3.7782 ± 0.291	—	2.4530 ± 0.394	—
5	0.5105 ± 0.047	2.16 ± 0.01	0.8176 ± 0.071	2.59 ± 0.01
15	0.0753 ± 0.003	9.18 ± 0.01	0.1546 ± 0.023	10.70 ± 0.24
30	0.0485 ± 0.006	15.37 ± 0.30	0.0626 ± 0.008	17.06 ± 0.12
60	0.0393 ± 0.007	20.54 ± 0.42	0.0433 ± 0.003	21.97 ± 0.08
90	0.0371 ± 0.002	24.11 ± 0.03	0.0410 ± 0.005	23.68 ± 0.54
120	0.0343 ± 0.007	26.21 ± 0.18	0.0398 ± 0.006	24.90 ± 0.32
180	0.0322 ± 0.004	28.37 ± 0.99	0.0386 ± 0.001	27.04 ± 0.25
240	0.0305 ± 0.002	29.73 ± 0.46	0.0374 ± 0.002	28.83 ± 0.71
360	0.0289 ± 0.003	31.71 ± 0.08	0.0363 ± 0.003	29.95 ± 0.52
qso	183153 dpm		170724 dpm	
	Black - C.S		Black - C.NPS	
0	100.0000	0.0000	100.0000	0.0000
1	1.9412 ± 0.210	—	4.5552 ± 0.010	—
5	0.2824 ± 0.080	2.51 ± 0.11	0.8909 ± 0.052	2.11 ± 0.02
15	0.0719 ± 0.007	10.06 ± 0.43	0.2351 ± 0.020	10.76 ± 0.16
30	0.0494 ± 0.003	17.76 ± 0.21	0.0853 ± 0.004	18.48 ± 0.01
60	0.0418 ± 0.001	19.61 ± 0.83	0.0595 ± 0.004	24.62 ± 0.00
90	0.0408 ± 0.002	22.52 ± 0.04	0.0577 ± 0.007	26.72 ± 0.01
120	0.0402 ± 0.005	27.76 ± 0.22	0.0564 ± 0.002	29.55 ± 0.14
180	0.0389 ± 0.005	30.17 ± 0.25	0.0540 ± 0.003	32.45 ± 0.14
240	0.0376 ± 0.003	32.98 ± 0.06	0.0528 ± 0.001	34.81 ± 0.20
360	0.0362 ± 0.001	35.97 ± 0.35	0.0509 ± 0.004	35.69 ± 0.01
qso	161556 dpm		162868 dpm	

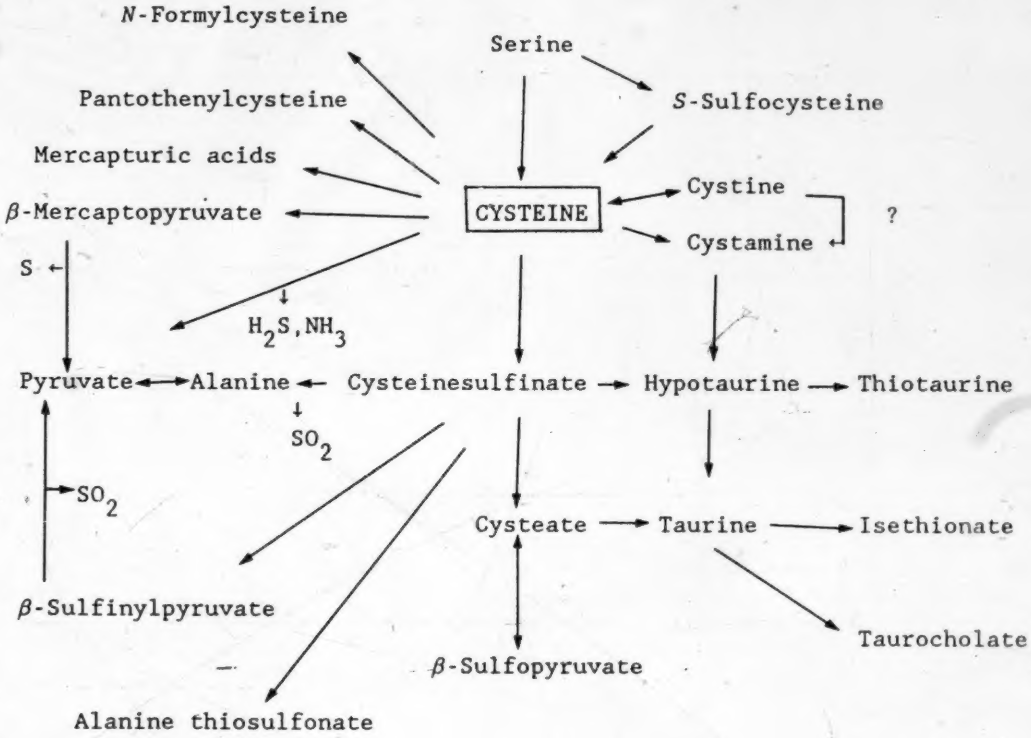
(continued...)

Time min	Black - C.Alfalfa		Black - C.Straw	
	Solution	CO_2	Solution	CO_2
0	100.0000	0.00	100.0000	0.00
1	3.4260 ± 0.216	—	4.5741 ± 0.721	—
5	0.5814 ± 0.051	8.29 ± 0.44	0.8822 ± 0.065	2.54 ± 0.15
15	0.1365 ± 0.010	21.06 ± 0.08	0.2459 ± 0.037	10.61 ± 0.39
30	0.0797 ± 0.002	25.43 ± 0.80	0.1408 ± 0.020	17.31 ± 0.70
60	0.0546 ± 0.007	30.03 ± 0.87	0.0923 ± 0.007	23.92 ± 0.98
90	0.0518 ± 0.009	33.30 ± 0.57	0.0736 ± 0.009	26.47 ± 0.18
120	0.0479 ± 0.003	34.00 ± 0.22	0.0654 ± 0.002	29.12 ± 0.35
180	0.0457 ± 0.007	35.88 ± 0.09	0.0537 ± 0.001	31.99 ± 0.11
240	0.0435 ± 0.008	37.99 ± 0.08	0.0514 ± 0.005	34.38 ± 0.93
360	0.0412 ± 0.001	39.36 ± 0.51	0.0496 ± 0.003	36.45 ± 0.14
qso	179216 dpm		171160 dpm	
	Black - C.Glucose		Black - C.Amino-acid mixture	
0	100.000	0.00	100.0000	0.00
1	4.9025 ± 0.100	—	3.6158 ± 0.350	—
5	0.5862 ± 0.121	2.94 ± 0.04	0.4537 ± 0.090	0.44 ± 0.00
15	0.0999 ± 0.008	6.83 ± 0.16	0.0927 ± 0.007	1.37 ± 0.08
30	0.0386 ± 0.007	9.74 ± 0.17	0.0392 ± 0.003	4.15 ± 0.01
60	0.0261 ± 0.004	15.94 ± 0.41	0.0328 ± 0.004	9.19 ± 0.04
90	0.0227 ± 0.002	18.74 ± 0.00	0.0318 ± 0.003	12.84 ± 0.47
120	0.0210 ± 0.006	21.82 ± 0.08	0.0307 ± 0.005	17.26 ± 0.48
180	0.0187 ± 0.000	24.48 ± 0.47	0.0291 ± 0.002	22.53 ± 0.00
240	0.0176 ± 0.001	26.26 ± 0.14	0.0275 ± 0.001	27.05 ± 0.26
360	0.0164 ± 0.003	28.68 ± 0.38	0.0265 ± 0.001	31.53 ± 0.22
qso	176032 dpm		188640 dpm	

(continued...)

Table 2. Number of organisms before and after sterilization of a Gray Luvisolic soil (2 year rotation).

	Number of Microorganisms g^{-1} soil			
	Control		Autoclaved	
Time (min)	Bacteria	Fungi	Bacteria	Fungi
0	$5.4x10^6$	$1x10^4$		
1			$1x10^3$	$<1x10^2$
10			$6x10^3$	$<1x10^2$
30			$4x10^3$	$<1x10^2$
60			$5x10^2$	$<1x10^2$
120			$1x10^3$	$<1x10^2$
240			$1x10^2$	$<1x10^2$
360			$2x10^3$	$<1x10^2$


N-Formylcysteine
Serine
S-Sulfocysteine
Pantothenylcysteine
Mercapturic acids
CYSTEINE
Cystine
?
β-Mercaptopyruvate
Cystamine
S
H2S,NH3
Pyruvate
Alanine
Cysteinesulfinate
Hypotaurine
Thiotaurine
SO2
SO2
Cysteate
Taurine
Isethionate
β-Sulfinylpyruvate
Taurocholate
β-Sulfopyruvate
Alanine thiosulfonate

Summary scheme for the metabolism of cysteine.
(Redrawn from Meister, 1965)

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*************CONTINUOUS SYSTEM MODELLING PROGRAM*******************
*****************PROBLEM INPUT STATEMENTS**** TWO MICROBIAL COMP*****
TITLE      AMINO ACID CYCLING IN SOIL SOLUTIONS (FOUR COMPONENT)****
*******************************************************************
INITIAL
NOSORT
************************ P A R A M E T E R S **********************
     PARAM K1=2.1
*K1 is adsorption rate (min,-1)
     PARAM K2=0.0086
*K2 is desorption rate
     PARAM K3=0.90
*K3 is microbial uptake rate
     PARAM K4=0.48
*K4 is anabolic rate
     PARAM K5=0.170
*K5 is respiration rate from cytoplasmic amino acid pool
     PARAM K6=.0005
*K6 is respiration rate from protein pool

********* I N I T I A L   V A L U E S   O F   I N T E G R A L *********
     PARAM IS=100
*IS  initial amount of C-14 in solution
     PARAM IA=0.0
*IA  initial amount of C-14 in adsorbed phase
     PARAM IM=0.0
*IM  init.  amt. C-14 in cyt. pool
     PARAM IP=0.0
*IP  init. amt of C-14 in prot. pool
     PARAM IO=0.0
*IO  init. amt. of C-14 as CO2 evolved from cyt. pool
     PARAM IQ=0.0
*IQ   CO2 evolved from protein pool

*******************************************************************
DYNAMIC
*******************************************************************

**************** B A L A N C E   E Q U A T I O N S ****************
DS=(K2*A*INSW(A,0.0,1.0))-((K1+K3)*S*INSW(S,0.0,1.0))
DA=(K1*S*INSW(S,0.0,1.0))-(K2*A*INSW(A,0.0,1.0))
DM=(K3*S*INSW(S,0.0,1.0))-((K5+K4)*M*INSW(M,0.0,1.0))
DP=(K4*M*INSW(M,0.0,1.0))-(K6*P*INSW(P,0.0,1.0))
DO=(K5*M*INSW(M,0.0,1.0))
DQ=(K6*P*INSW(P,0.0,1.0))
*******************************************************************
********************** I N T E G R A L S *************************
     S=INTGRL(IS,DS)
     A=INTGRL(IA,DA)
     M=INTGRL(IM,DM)
     P=INTGRL(IP,DP)
     O=INTGRL(IO,DO)
     Q=INTGRL(IQ,DQ)
     R=O+Q
     T=S+A+M+P+O+Q
*******************************************************************
TERMINAL
TIMER  FINTIM=360.0,PRDEL=1.0,OUTDEL=1.0,DELT=0.001,DELMIN=2.0E-8
METHOD MILNE
RELERR  S=0.00001, A=0.00001, M=0.00001, P=0.00001, O=0.00001, Q=0.00001

PRTPLOT S,A,M,P,O,Q,R,T
END
STOP
ENDJOB
```

```
**************CONTINUOUS SYSTEM MODELLING PROGRAM*******************
*****************PROBLEM INPUT STATEMENTS****    (4 components)
TITLE      AMINO ACID CYCLING IN SOIL SOLUTION~~S~~ (STABILIZATION)
*******************************************************************
INITIAL
NOSORT
************************* P A R A M E T E R S ********************
     PARAM K1=6.50
*K1 is adsorption rate (min,-1)
     PARAM K2=0.008
*K2 is desorption rate
     PARAM K3=1.870
*K3 is microbial uptake rate
     PARAM K6=0.00250
*K6 is microbial-2 oxidation
     PARAM K7=0.0005
*K7 is colloidal stabilization
********* I N I T I A L   V A L U E S  O F  I N T E G R A L *********
     PARAM IS=100
*IS  initial amount of C-14 in solution
     PARAM IA=0.0
*IA  initial amount of C-14 in adsorbed phase
     PARAM IM=0.0
*IM  init.  amt. C-14 in microbial pool
     PARAM IP=0.0
*IF  init. amt of C-14 in stabilized pool
     PARAM IO=0.0
*IO  init. amt. of C-14 as CO2 evolved from microbial pool
******************************************************************
DYNAMIC
******************************************************************

**************** B A L A N C E  E Q U A T I O N S ****************
DS=(K2*A*INSW(A,0.0,1.0))-((K1+K3)*S*INSW(S,0.0,1.0))
DA=(K1*S*INSW(S,0.0,1.0))-((K2+K7)*A*INSW(A,0.0,1.0))
DM=(K3*S*INSW(S,0.0,1.0))-(K6*M*INSW(M,0.0,1.0))
DF=(K7*A*INSW(A,0.0,1.0))
DO=(K6*M*INSW(M,0.0,1.0))
******************************************************************
*********************** I N T E G R A L S ************************
     S=INTGRL(IS,DS)
     A=INTGRL(IA,DA)
     M=INTGRL(IM,DM)
     F=INTGRL(IF,DF)
     O=INTGRL(IO,DO)

     T=S+A+M+F+O
******************************************************************
TERMINAL
TIMER  FINTIM=360.0,PRDEL=1.0,OUTDEL=1.0,DELT=0.001,DELMIN=2.0E-8
METHOD MILNE
RELERR  S=0.00001, A=0.00001, M=0.00001, F=0.00001, O=0.00001
PRTPLOT S,A,M,F,O,T
END
STOP
ENDJOB
```